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Immunization Against Pox in Domestic Fowl

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UNIVERSITY OF ILLINOIS
AGRICULTURAL EXPERIMENT STATION

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Immunization Against Pox In Domestic Fowl

By ROBERT GRAHAM and C. A. BRANDLY*

FOR MANY YEARS fowl pox has caused serious losses in Illinois farm flocks. Lowered egg yield, impaired development, and loss of flesh, together with high death rate, as the result of the generalized type of the disease, have been noted. In fact, pox often recurs on many premises, and in some districts has even assumed endemic proportions. The predominating type of the disease in chickens in Illinois is characterized by the development of diphtheritic patches on the mucous membranes of the mouth, while in turkeys the infection is characterized by epithelioma-like lesions of the comb and wattles.

Fowl-pox immunization procedures have been studied at the Illinois Agricultural Experiment Station since 1926. In these studies an opportunity has been afforded to employ different pox viruses in appraising the limitations of immunization. To aid flock owners and veterinarians in the control of pox in domestic fowl, suggested methods were outlined in Circular 430 (1935). The monograph presented here gives a partial exposition of the results of fowl- and pigeon-pox immunization studies at the Station from 1926 to 1936, together with a review of available literature on fowl-pox immunization.

Investigations on pox control in domestic fowl at the Illinois Station have centered about the development of an active virus that might consistently induce immunity, yet be incapable of inducing unfavorable reactions. Numerous favorable reports on pox control by artificial immunization have appeared in the world's literature during the past quarter of a century. These investigations, tho conducted

NOTE.—The term fowl pox, as employed here, refers to a group of so-called pox infections which attack various species of Aves, accompanied by the syndrome and lesions of the character considered grossly or microscopically typical of avian poxes. A distinction is made between strains of fowl-pox and pigeon-pox virus. Fowl-pox virus (also called fowl virus) usually attacks only chickens and turkeys. Pigeon-pox virus (also referred to as pigeon-strain virus or pigeon virus) is common only to pigeons, and, like fowl virus, is distinct from other bird-pox viruses such as Kikuth's canary-pox virus and mourning-dove virus.

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in different countries under a variety of conditions and with different strains of fowl- and pigeon-pox viruses have, in the main, reported progress in the suppression of pox with modified active virus; yet certain limitations are generally acknowledged in immunization procedures. The investigations at the Illinois Station, herein reported, were prompted by limitations encountered in employing viable fowl and pigeon viruses with the object of more completely eliminating irregular or unfavorable results in pox prophylaxis.

Prior to 1902 efforts to find a dependable method of immunizing susceptible fowls against pox were largely fruitless. These failures may probably be attributed to the confusion which existed regarding the etiology of the disease. Hopeful progress was not recorded in immunization against pox in domestic fowl until it was recognized that cutaneous pox (so-called epithelioma) and the mucous-membrane type of the infection (diphtheria) were caused by the same infective agent. The identification of the common etiology of the two types of the disease was followed by convincing evidence that inactivated or killed pox virus failed to stimulate more than a transient or negligible pox immunity. This information prompted extensive studies on the immunizing value of viable pox virus; and, as a result of applying this virus in various ways, the dermatropic character, as well as the immunizing value, of living pox virus was established.

PART I: REVIEW OF LITERATURE

As early as 1869 Rivolta described "inclusion" bodies as etiologic factors of fowl pox. Later (1880) the same author ascribed mycotic characters to the "inclusion" bodies and reported flagellates associated with diphtheria in young fowls and pigeons. Silvestrini (1873), Peroncito (1886), Pfeiffer (1889), Babes and Puscaru (1890), and Mazanti (1896) ascribed causal significance to various protozoa encountered in diphtheritic or pox-like lesions of chickens and other birds. Bollinger (1873) called attention to the microscopic changes in fowl-pox lesions. Later Krajewski (1887), Babes and Puscaru (1890), Loir and Ducloux (1894), Sanfelice (1897), Harrison and Streit (1902-04), Fally (1908), Jowett (1909), Bordet and Fally (1910), Gallio-Valerio (1925), and others reported bacteria, yeasts, and molds in the role of causative agents of avian diphtheria and pox-like lesions in various birds.

The various conceptions of the etiology of pox were not clarified until Marx and Sticker (1902) demonstrated that the etiologic factor of fowl pox was capable of passing a Berkefeld filter. The filtrable character of the etiologic factor, together with evidence that specific

cytologic changes were produced by the filter-passing agent, was accepted as identification of fowl pox as a virus disease. Subsequently Carnwarth (1908), Von Betegh (1913), Von Ratz (1913), and Van Heelsbergen (1920) demonstrated the etiological identity of cutaneous fowl pox and avian diphtheria, while Panisset and Verge (1923) described pox lesions on the skin, the mouth, and the oculonasal membranes and found them all to be associated with the virus of pox. Doyle and Minett (1927) confirmed these observations and, in addition, called attention to so-called "no-lesion" cases of fowl pox characterized by chronic emaciation; while the observations of Borrel (1904) on the morphological structure of pox inclusions were followed by studies of Woodruff and Goodpasture (1929) establishing pathogenesis of a single elementary (Borrel) body.

The early reports on the use of "vaccines" prepared from pox-lesion material treated by chemical and physical means suggest that these products in many cases were not true vaccines but suspensions of inactive or dead virus rather than the attenuated active virus. Variations in the effect of the "attenuating" agent due to temperature, nature, and concentration of chemicals employed, to the virus as well as the concentration of the virus, bacterial flora, etc., might account for marked differences in antigenic properties. When the chemical or physical treatment of the lesion material was so severe as to inactivate completely or destroy the virus, the tissue and immunity responses appeared negligible. In cases where chemical or physical treatment resulted in attenuation by inactivation or destruction of only a part of the pox virus, a "true" antigenic vaccine was apparently obtained. Such pox vaccines appeared capable of inducing a localized reaction recognized as a vaccination infection or "take" in susceptible birds. Such reaction, in view of present knowledge, is followed by an active-immunity response.

In many investigations on immunization procedures the actual potency or infectivity of the virus content of the vaccines was not determined, and it seems obvious that an accurate appraisal of antigenic properties thus would be impossible. In numerous instances physical and chemical attenuation of a significant degree probably occurred in the preparation and handling of the virus, altho this alteration was not fully recognized. Furthermore, some reports fail to indicate definitely the source of the pox virus employed, designating it as fowl-pox virus, altho it appears that virus of pigeon source might have been employed. Biological variation and strain differences, particularly in so far as various fowl and pigeon strains are concerned, were in some instances apparently overlooked or incorrectly interpreted. Furthermore, vaccines were often employed in pox-infected flocks; and the results, if any, could not be ascertained accurately.

In an attempt to analyze fowl-pox immunization procedures Reiz and Nobrega (1936) classified the vaccines into four principal types according to source or origin; namely, original chicken (unmodified fowl pox) virus; original (unmodified) pigeon virus, monopathogenic; chicken (fowl) virus (bipathogenic) adapted to pigeons; and mixed virus of the chicken and pigeon. To these may be added pigeon virus (bipathogenic) unmodified, or modified by serial chicken passage, particularly since monopathogenic pigeon strains may not be considered pathogenic or antigenic for chickens. More recent findings suggest that a classification of original or unmodified, as well as biologically modified, strains may warrant recognition, tho earlier workers employed principally the unmodified fowl-pox lesion material treated by chemical and physical means, with the object of bringing about a satisfactory degree of attenuation. Later, virulent unmodified fowl- and pigeon-pox viruses were widely used, while attempts to effect desirable modification of bipathogenic viruses by passage thru the heterologous species have received attention.

Chemically and Physically Treated Virus

The non-antigenic properties of dead fowl virus were reported by Burnet (1906), Beach (1920), Bierbaum, Eberbeck, and Rasch (1929), and Kligler (1930), while Doyle (1930) reported that fowl virus modified or attenuated by chemical means produced only inconstant results. Manteufel (1910) reported success in immunizing fowls by injecting subcutaneously and intravenously lesion virus from the skin or mucous membranes, suspended in physiological salt solution and heated at 55° C. for one hour. Successful attenuation of the virus by admixture with rabbit bile was also claimed. Manteufel further claimed marked curative value for this product and stated that chickens treated with it were immune for one and one-half to two years. Hadley and Beach (1913), Mack and Records (1915, 1916), Upton (1918), Klose (1921), and Glover (1931) concluded that vaccine of the Manteufel type was highly effective in preventing fowl pox and exerted a curative effect on diseased fowls. Immunity was obtainable by subcutaneous injection of pox material, both heated and unheated.

However, Boerner and Stubbs (1921), as well as McNutt (1926), Pyle (1926), and Bierbaum, Eberbeck, and Rasch (1929), failed to obtain satisfactory results in the control of outbreaks of fowl pox by means of heated vaccines of the Manteufel type and concluded they were of little value. The use of fowl-pox vaccines of this type in controlling outbreaks of pox was regarded favorably by Fuller (1923, 1924) and Gwatkin (1925). Panisset and Verge (1923) inoculated intradermally the barbs of the comb, using .1-cc. quantities of skin virus

treated with .5-percent phenol. The local or cutaneous immunity induced lasted at least four months. Pyle (1926) found only a poor or low grade of immunity of very limited duration after as many as three subcutaneous injections with several heat-treated commercial fowl-pox vaccines; while Bierbaum, Eberbeck, and Rasch (1929) concluded that no immunity was obtainable from the use of virus weakened by formalin or carbolic acid.

According to Beach (1929), .1-percent formalin, 1-percent liquid chloroform, and the passage of chloroform vapor thru the fowl-pox vaccine destroyed it rapidly; while Kligler (1930) concluded that fowl-pox virus relatively free of protective protein when heated to 56° C. for one hour or treated with .5-percent formalin solution for four days no longer produced lesions in susceptible chickens and also failed to induce immunity. Phenolized vaccine (.25-percent phenol) still contained active virus fifty days after preparation; the survival of virulence depended on the concentration of the virus suspension. One injection of a phenolized vaccine which no longer produced active lesions was sufficient to produce immunity. Heated phenolized virus failed to produce immunity, thus indicating that the immunity obtained with the unheated phenolized vaccine was induced by the surviving live virus.

Glover (1931), in a survey of work upon heated and chemically treated fowl-pox vaccines, concluded that two inoculations with virus suspensions rendered inert by heat, formalin, or chloroform are capable of inducing only a fleeting immunity which was weakened by the eighth week and disappeared entirely by the twenty-fourth week following treatment. Phenol appeared less injurious to fowl-pox virus than various other chemicals, according to Kligler and Olitzki (1931), in both "protein-containing" and "protein-free" preparations of fowl-pox virus. The virus was apparently much less sensitive to the action of phenol and ether than to other agents, such as formalin, mercuric chlorid, hydrogen peroxid, and sodium bisulfite.

In this connection Zwick (1930) pointed out that when .5-percent phenol is added to fowl-pox virus it may "weaken" the virus but still leave it antigenic. With this product a sufficient amount of virus may survive for a protracted period to induce a high degree of resistance, but there still remains the danger of "inoculation pox." In a comparative study of pigeon-pox and phenol-attenuated fowl-pox vaccines, Kligler, Komarov, and Fiat (1933) found that cutaneous vaccination with the fowl-pox vaccine produced lesions of longer duration and was often associated with secondary lesions in the mouth and comb. The risk of secondary lesions with the fowl-pox virus was said to be relatively small in healthy birds but more serious in unhealthy flocks. The duration as well as the degree of immunity produced by the attenuated fowl-pox vaccine was found to be greater than that pro-

duced by pigeon vaccine. Morcos (1931) reported that pigeon-pox virus treated with .25-percent formalin and kept at room temperature for two days protected both pigeons and fowls against the homologous virus. Komarov and Kligler (1936) obtained results with phenol-treated fowl-pox virus which confirmed the limitations previously observed and, in addition, suggested the inadvisability of using fowl-pox vaccine in flocks of late hatch. Picard (1931, 1931A), working with formalized fowl-pox vaccine in the Dutch East Indies, reported unsatisfactory results.

According to Goodpasture (1928), Lipschutz found that 1 percent of saponin had no effect in one hour but after 24 hours there was apparently some diminution in infectivity of the virus. Graham and Barger (1936) added 2 percent of saponin to a 1-percent aqueous suspension of fowl-pox virus which was used for cutaneous vaccination of chickens. The infectivity of the virus suspension was destroyed by the saponin after 45 hours at 12 to 15° C., and the feather-follicle method of application of this material did not protect against artificial exposure.

Virulent Fowl Virus

Recognition that the immunizing value of so-called pox vaccines attenuated by chemical and physical means, as reported in numerous independent investigations, was subject to wide variations, directed attention of investigators to the use of fresh pox-lesion material not treated with chemical or physical agents. De Blicke and Van Heelsbergen (1923) were perhaps the first to employ, on a significant scale, fully virulent fowl-pox virus for cutaneous vaccination against fowl pox. The virus was applied to the scarified skin in a manner similar to Jennerization in man. Basset (1924) inoculated unattenuated fowl-pox virus into the pectoral muscles of fowls and reported the production of a satisfactory immunity. This route was favored over intracutaneous or subcutaneous introduction, and Basset (1924) stated that only local lesions were induced unless the dosage of virus was very large. Waite (1924) applied live pox virus in the form of comb-lesion material to a small scarified area of comb and reported a high percentage of takes with apparent immunity as determined by absence of subsequent outbreaks of pox. Beach (1927) claimed fowls were immunized against pox by subcutaneous injection of fresh lesion and sublesion as well as other tissue, from artificially infected cockerels, suspended in a mixture of equal parts of glycerin and 1-percent phenolized saline. It was inferred that attenuation of the virus was not necessary to make the vaccine safe for use and that the development of lesions at the point of inoculation of the virus was apparently not essential to the production of immunity. Johnson (1927) used unattenuated virus applied to a scarified area of defeathered skin or

to open feather follicles and concluded that fowl-pox virus vaccination may be successfully used in commercial flocks to prevent fowl pox. Weaver (1927) inoculated dried fowl-pox virus into 6 to 10 feather follicles of the skin of the legs of pullets. Danger of generalization of pox from vaccination was observed. Upon contact exposure after 6 weeks all showed a high degree of immunity. Sawyer (1928) stated that "fowl pox virus vaccination by the feather follicle method produced no apparent bad results in fowls three to four months old and the immunity lasted at least two years." Pyle (1928) concluded that a marked local skin response or take following vaccination was necessary in establishing a high degree of cutaneous immunity. Edgington and Broerman (1928) reported that results from vaccination with fresh fowl-pox scabs applied by skin scarification or by the removal of 1, 5, or 10 feathers appeared about the same. A measurable immunity which prevailed for at least four months was induced in birds showing a satisfactory take. The so-called stick, or puncture, method of cutaneous vaccination with fowl-pox virus was advocated by Johnson (1929) with the object of overcoming certain disadvantages of the feather-follicle method; that is, irregular or excessive dosages and loss of time and material.

Results indicating that cutaneous vaccination of chickens with living fowl virus was in general quite satisfactory were reported subsequently by Beach (1929), Beaudette (1929), Gildow and Bottorff (1929), Gildow, Schilling, Moore, and Lampman (1929), Jones (1929), Sawyer (1929), Smith (1930), Stafseth (1930), King and Trollope (1930), Glover (1930), and others. Altho the results of this and subsequent work revealed that vaccination of chickens with unmodified fowl-pox virus could be relied upon to produce a high grade of immunity which persisted for an extended period of time, certain potential hazards were emphasized. Severe post-vaccination reactions manifested by constitutional disturbances, as well as considerable mortality, were frequently encountered in flocks suffering concurrently from parasitism and other diseases, according to Johnson (1927) and Stafseth (1931). Factors of environment and management tending to affect adversely the vitality of the flock have also been found to favor undesirable reactions subsequent to active virus vaccination. In fact, serious impairment of production almost universally contraindicates vaccination of laying flocks with unmodified fowl-pox virus.

Johnson (1927) and other workers recommended that cutaneous vaccination of young stock with unattenuated fowl virus be carried out during the summer and early fall when weather conditions were generally more favorable and the birds were three to five months old. The importance of vaccination at least several weeks prior to the onset of production, to avoid serious interference therewith, was recognized by Edgington and Broerman (1928), Beach (1929), and Glover

(1930). The minimum age at which vaccination could be carried out safely was generally accepted as being about four weeks, tho Johnson (1929) suggested that fowls probably showed a less marked systemic effect after the age of three months. However, Johnson (1930) reported satisfactory results in vaccination of 30-day-old chicks by the stick method. According to Sawyer and Hamilton (1930), the results of a questionnaire on cutaneous vaccination with unattenuated fowl virus in the state of Washington during the preceding season suggested that the use of fowl-pox virus vaccine was justified on three- to four-month-old chickens.

Pyle (1929) observed that cutaneous vaccination did not cause a retardation of weight gains on birds treated at 80 days of age or older, but that weight gains were slightly retarded in birds 68 days of age. The results of a careful study of the effect of unmodified fowl-pox virus vaccination on chickens of different ages were reported by Lubbehusen and Ehlers (1932). Groups of birds were vaccinated by the feather-follicle and stick methods at ages ranging from 30 to 156 days. The conclusion was reached that "vaccination of birds between 30 to 90 days is to be recommended, since the systemic reaction incident to vaccination during this period does not appreciably affect normal growth and development." In birds vaccinated between 90 and 120 days of age, normal weight gains were inhibited while vaccination of birds older than 120 days produced a distinct post-vaccination shock. Danks (1931) conducted experiments with a small number of chickens and turkeys to ascertain whether very young chicks could be successfully vaccinated. A 2-percent suspension of virulent fowl-pox scabs (powdered) in liquid paraffin was used to inoculate plucked areas as well as plucked and scarified areas on the thighs of 1- to 8-day-old chicks and turkey poults. Vaccination takes were produced consistently, and complete immunity to artificial exposure was demonstrated at two months but not at three months after vaccination. Since generalization and considerable mortality occurred in one lot of chicks after vaccination, Danks concluded that it is inadvisable to vaccinate chicks less than one month old except under unusual circumstances.

Subsequently Sherwood (1932) reported the vaccination of day-old chicks and 2-week-old turkey poults with virulent fowl-pox virus by a combined feather-follicle and scarification method. Later Dunn and Sherwood (1933) concluded that healthy, vigorous, day-old chicks and poults can be safely and successfully vaccinated against fowl pox with fowl-pox virus (1 part to 250 parts of 40-percent glycerin-saline) without causing an apparent constitutional disturbance. Slight scarification of the plucked skin was deemed necessary to effect successful inoculation because the feather follicles are quite small at this age. Seddon, Hutcheson, and Murphy (1932) reported excellent results

from field vaccination work in Australia extending over three seasons. Birds 6 to 20 weeks of age were vaccinated, and it was concluded that, other conditions being favorable, the optimum age for vaccination was between 12 and 16 weeks. They used freshly collected scabs from fowl-pox comb lesions, dried at 37° C. for 24 hours, then powdered and stored for four to twelve months in the refrigerator. A .1-percent virus suspension in saline was used. However, in the observations of Zwick (1930) and others the potentialities of generalized inoculation pox could not be eliminated under all circumstances with active virus. Hinshaw (1933) stated that "observations made over a period of four years have shown that turkeys have none of the post-vaccination difficulties often observed in chickens."

Bice (1933) concluded from the results of three and one-half years' experience with fowl-pox virus vaccination in Hawaii that the proper age for vaccinating chickens was 4 to 12 weeks. Heavy mortality occurred following vaccination of younger birds by the routine method, while the vaccine was considered unsuitable for adult fowl. He also reported the successful vaccination of turkey poults ranging from 3 to 16 weeks of age, but used a smaller knife for the stick vaccination and a different dilution of the virus. The importance of proper nutrition and management, including protection against mosquito vectors prior to and for three weeks following vaccination, was emphasized. Martin (1933) concluded, from vaccination of chickens with unattenuated fowl virus by the feather-follicle method over a seven-year period, that a solid life protection was induced by inoculating 3 to 6 feather follicles on each bird. Vaccination was seldom fatal, but mature birds which had not molted were thrown into a molt and production in pullets was greatly reduced. Johnson (1934) considered vaccination with fowl virus very successful for turkeys and chickens not in production.

Coronel (1934) employed for vaccination of chickens a strain of pox virus from the turkey which, when first collected, was virulent for chickens but after two months at 0 to 5° C. was said to be sufficiently attenuated not to cause serious generalized infection and at the same time conferred immunity. He suspended 11 mg. of the virus in 2 cc. of saline and made a single scratch scarification on the comb with a knife previously dipped in virus. Brunett (1934) employed virulent pox virus from the chicken, turkey, and pigeon to vaccinate turkeys by the follicle method. A marked focal reaction, or take, was obtained from all viruses but no generalized effect was observed. The chicken and turkey viruses gave immunity, but no apparent immunity was induced with the pigeon virus against the turkey or chicken virus. Lubbehusen and Ehlers (1934) observed from comparative vaccination experiments with fowl and pigeon virus that: "Altho its immunizing efficiency is unquestioned, fowl-pox vaccination has definite

limitations. Birds should be vaccinated when the systemic reaction incident thereto is less apt to be followed by undesirable sequelae." Basset (1935), in reporting further on vaccination of fowls with virulent fowl-pox virus, recommended inoculation with a measured dose of the homologous virus into the pectoral muscles.

Brandly (1936) found fowl-pox virus propagated in pure culture in the developing chicken egg a satisfactory substitute for the comb- or skin-lesion virus in common use for vaccination of chickens. Brandly and Dunlap (1939) found that pigeon- and fowl-pox viruses grown *in vitro* in tissue cultures also were suitable for use in immunization of chickens. In a preliminary report on fowl-pox virus vaccination in day-old chicks, Lubbehusen, Beach, and Busic (1936) stated:

"The controlled experimental data indicate that a vaccination take, even in vigorous day-old chicks, is accompanied by a systemic reaction which manifests itself by at least a temporary inhibition of normal weight gains and a lowering of vitality, the degree of which is influenced by the severity and duration of the local reaction and which in the presence of unfavorable environment and concurrent disease may contribute to excessive mortality. . . . In measuring the post-vaccination reaction in terms of growth gains and mortality, there are indications that this reaction occurs but that it is less pronounced in chicks vaccinated at the age of three weeks and is entirely absent at eight weeks."

Contact exposure of vaccinated day-old chicks indicated an adequate resistance for at least five months (the longest period tested), but the degree of reaction to artificial exposure suggested that the immunity in these chicks was less stable than that in the chicks vaccinated at a later date. In a second report Lubbehusen and Beach (1937) confirmed the observations regarding post-vaccination reactions in chicks vaccinated at one day of age and stated that their data "also indicate that a systemic reaction followed vaccination at the ages of 13, 21, 28, and 42 days but it was of slower onset and progressively less severe as the age increased than that observed in day-old vaccinated groups."

In both of the immediately foregoing reports attention is directed to the results which indicated that vaccine concentrations greater than 10 mg. of powdered virus material per cc. were undesirable, sometimes causing unnecessarily severe reactions. Vaccination was done by the stick method in the skin of the flank, and the special instrument recommended for this procedure was said to insure application of uniformly small amounts of virus. Komarov and Kligler (1936) in Palestine concluded from the results of an experiment on the effect of age on the incidence of secondary lesions: ". . . it is apparent (a) that it is dangerous to vaccinate baby chicks with fowl-pox vaccine; (b) that healthy birds, two to three months of age may be vaccinated with fowl-pox vaccine without serious risk." From the results of comparative tests of vaccination in early and late hatches

of chickens the same authors also stated that "it becomes obvious that fowl-pox vaccine cannot be recommended for use in flocks of late hatch."

Virulent Pigeon Virus

In 1926 Saito drew attention to the immunological relationship between fowl-pox and pigeon-pox viruses, suggesting that either type of virus produced immunity in both species of birds. Doyle and Minett (1927), unaware of Saito's work, made similar observations. Zwick, Seifried, and Schaaf (1928) reported that vaccination of chickens with pigeon-pox virus gave, after three weeks, immunity to artificial and natural infection with fowl pox. With pigeon virus unfavorable results such as generalization and death were not encountered. Lahaye (1928) vaccinated thirty fowls with pigeon virus and later found that they were protected against artificial and natural infection with both fowl and pigeon strains of pox virus.

Bierbaum, Eberbeck, and Rasch (1929) reported that pigeon-pox virus was capable of inducing considerable resistance against fowl pox. Doyle (1930) found that pigeon-pox virus conferred a solid immunity against natural infection with fowl pox and that the immunity was fully established in 14 days after inoculation and persisted for about six months. Considerable but not complete protection was obtained to artificial fowl-pox exposure. W. T. Johnson (1930, 1931), in comparative tests of fowl- and pigeon-pox virus, observed that fowl-pox virus caused a drastic drop in production during the three to four weeks after vaccination, while other pullets in the same flock receiving pigeon virus showed only a slight reduction during the same period. Lerche (1931) reported that pigeon virus was effective for immunization of chickens by cutaneous vaccination. Danks (1931) recorded little, if any, protection against subsequent fowl-pox inoculation exposure following vaccination with a 2-percent suspension of pigeon virus in liquid paraffin. Glover (1931) reported that pigeon-virus vaccination protected fowls against severe artificial exposure to fowl pox two months later. Stafseth (1931) recommended that pigeon-pox virus be used for vaccination of mature birds because of the danger of systemic reactions with fowl-pox virus. Ramazzotti (1931) concluded that injection of the fowl with pigeon-pox virus confers an immunity to fowl pox. Cominotti and Pagnini (1931) claimed from their researches that pigeon-pox virus proved an excellent material for vaccinating chickens against fowl pox, the resulting immunity enduring for longer than one year. In healthy fowls the local reaction resulting from intradermal inoculation of pigeon virus was never followed by generalization. In some infected birds vaccination may cause a fibrinous exudate on the oropharyngeal mucosa attributed to the action of homologous virus which, under considerations of succes-

sive allergic reactions to heterologous virus, passes from a latent state to a pathogenic explosion at the elective sites. These localizations of homologous virus are, in the majority of cases, of brief duration and usually tend to rapid dissolution without repercussion (emaciation) on the general state of the organism.

E. P. Johnson (1931, 1932, 1932A) reported that vaccination with pigeon-pox virus of chickens in the field conferred resistance to natural exposure to fowl pox for at least one year, and there was no systemic reaction or decrease in egg production. However, it was not 100-percent efficient in protecting cockerels against artificial exposure. W. T. Johnson (1931) noted a marked improvement in health of hens affected naturally with fowl pox after they had been treated with pigeon-pox vaccine. Canham (1932) observed from experimental work that pigeon-pox vaccine in 1-percent suspension in 80-percent glycerol-saline produced immunity against natural infection but only a partial immunity against artificial infection with fowl pox. It was found that birds a week old could be vaccinated safely with pigeon virus. None of the birds was observed to show any constitutional symptoms.

In tests on commercial pox vaccines for chickens the California Agricultural Experiment Station (1933) found that the pigeon-pox virus vaccines did not protect chickens against fowl pox. Michael (1932) reported the results of experiments in which increased resistance of fowl to natural or artificial infection could not be demonstrated after vaccination with pigeon-pox virus by the feather-follicle method. Crawford (1932) found that a pigeon-pox vaccine for the prevention of fowl pox in chickens was well adapted to Ceylon, while Broerman and Edgington (1932) vaccinated chickens with 1-percent suspensions of powdered pigeon-virus scabs by inoculating one "stick" and one feather follicle. Most fowls were not immune to severe fowl-pox exposure 90 days later, and 60 percent of the fowls exposed to natural fowl-pox infection four months later contracted the disease. Pyle (1932) stated:

"Fowl-pox vaccine (prepared from pigeon-pox virus) does have an important use in preventing the spread of pox and canker in a flock of chickens in which the disease has appeared.

"The vaccine made from pigeon pox can be administered to fowls in full lay without any subsequent diminution in egg production other than that caused by mere handling of the birds. Neither will it cause any loss in condition nor produce any constitutional disturbance. But until such time as more is known about the degree of protection which it produces, its use should be confined to preventing the spread of the infection in a flock, especially if the birds are in a state of production."

Brunett (1933) found that pigeon-pox vaccine failed to protect fowls against artificial infection, altho some degree of resistance to severe natural exposure was manifested, and that pigeon-pox virus does not disturb egg production, while field observations made by

Brunett indicated that pigeon-virus vaccine has value in checking pox among mature laying chickens. He concluded that "pigeon virus has a place in the control program—it remains to learn how and when to use it." Orr and Emmel (1933) vaccinated fowls in an egg-laying contest with pigeon-pox virus by the stick method. No decrease in egg production after vaccination was observed and, in comparison with previous years, the incidence of fowl pox was greatly diminished. Bayon (1933) concluded from a review of the work of others that pigeon-pox vaccine gives uniformly good results, and that by vaccination with this agent fowl pox can be checked for a period of six to twelve months. Delaplane and Stuart (1933) tested a commercial pigeon-pox vaccine, employing the feather-follicle method of vaccination. Chickens vaccinated at 6, 8, 10, 12, and 14 weeks of age proved susceptible to artificial and natural fowl-pox exposure when tested four months later. Kligler, Komarov, and Fiat (1933) in Palestine compared the results with pigeon- and fowl-pox virus vaccines and concluded that the former does not involve the risk of secondary lesions. The duration and degree of immunity produced with attenuated fowl-pox virus was found to be greater than that secured with pigeon virus. With the latter, immunity was less solid and approximately 10 percent of the birds remained unimmunized, as judged by the occurrence of fowl pox in field flocks during four to six months subsequent to vaccination.

Dalling (1933), in discussing fowl-pox vaccination, pointed out the superiority of pigeon virus over fowl virus under prevailing conditions in England as a means of avoiding certain dangers incident to the use of the latter, but suggested that if fowl-pox virus is "weakened" somewhat a highly efficient and satisfactory vaccine may be produced. Furthermore, this investigator suggested that the results of further work will probably cause such a vaccine to be largely used in England. From the results obtained in applying pigeon-pox virus (Illinois strain) to 5,000 chickens one to three months old, Graham and Barger (1935) concluded that pigeon virus is harmless; that the feather-follicle method of inoculation (8 to 10 follicles) is preferred; that the stick method is of no value; and that only a partial resistance to fowl pox is induced by feather-follicle application, as judged by artificial exposure, while the duration of the modified protection was not determined. In a subsequent report (1936) evidence of some protection was noted in chicks vaccinated at 4 to 14 weeks of age when they were exposed to severe artificial infection at periods as long as six months after vaccination. Edgington (1934) cited additional experiments which he interpreted ". . . to confirm observations previously reported; viz., that the immunity conferred by pigeon-pox vaccine was not so complete as that resulting from vaccination with fowl-pox vaccine." In comparative experiments with fowl- and pigeon-pox viruses Lubbe-

husen and Ehlers (1934) observed that "pigeon-pox virus vaccination does not produce an immunity sufficiently adequate to warrant its exclusive use in the control of fowl-pox infection. It may be substituted for fowl-pox virus as a vaccine when a short-interval protection is desired and where the advantages of a less pronounced systemic reaction outweigh the potential hazards of inadequate protection against fowl-pox infection." Johnson, in the same year (1934), stated: "It is apparent from results in America that pigeon-pox virus cannot be recommended for vaccination in this country as a sole source of pox control, if at all."

Bierbaum (1935) reported that in vaccination of a limited number of three-month-old chickens with two different pigeon-pox vaccines, part of the birds were completely resistant and the balance partially so, when tested one, three, six, and twelve months later. His results indicate that the degree of resistance had diminished progressively with each succeeding test interval. Gaede (1935) inoculated cockerels cutaneously and intramuscularly with large doses of pigeon virus. He reported that only a partial immunity was produced against subsequent inoculation with pigeon virus, whereas the fowl-virus vaccination conferred a solid and lasting immunity to the homologous virus. Basset (1935) stated that if pigeon virus is inoculated into fowls on a large area (of skin) a complete immunity results, but that this sort of inoculation is not practicable. He considered that the immunity produced is in direct proportion to the pathogenicity of the vaccine.

From their work in Palestine, Komarov and Kligler (1936) concluded that in order to protect chickens against the ravages of pox they must be vaccinated as soon as possible after hatching. Their experience with pigeon-pox virus emphasized that vaccination would confer protection against natural outbreaks for periods of only five to six months, and hence it was tentatively concluded that vaccination with pigeon-pox virus at least twice a year would most likely reduce the incidence of the disease.

Pigeon and Fowl Viruses Passed Thru Heterologous Host

The serial passage of fowl- and pigeon-pox viruses thru heterologous hosts has been attempted by various workers as a means of studying the relationship of strains of bird-pox viruses, as well as with the object of bringing about desirable modification which might enhance their value for immunization purposes. Lahaye (1927) stated that pigeon virus passed thru fowls did not undergo attenuation and would still produce typical lesions in the pigeon. Doyle and Minett (1927), altho unsuccessful in previous attempts, were able to adapt a fowl strain of virus to the pigeon. Lesions were not produced in pigeons of the first passage but the exposed skin from these birds killed on the

sixth day was ground and applied to the plucked skin of a second series of pigeons. Slight swellings of the feather follicles were obtained. There was a progressive increase in size of the swellings with subsequent passages; and, from the fifth passage on, the lesions were well defined and similar to those seen in fowls inoculated by the same route. Doyle and Minett (1927) also observed that the passage of pigeon virus thru the fowl causes a change which prevents ready readaptation to the pigeon. Zwick, Seifried, and Schaaf (1928) and Zwick (1930) reported that fowl-pox virus passaged repeatedly in pigeons became attenuated for the chicken or lost its virulence entirely and, when used as a vaccine, stimulated an immunity that was similar in nature and duration (about one year) to that induced by the pigeon virus.

Doyle (1930) passaged pigeon-pox virus by comb inoculation thru nine series of fowls at 10-day intervals. As a rule, the lesions of the first few passages were scanty, but there was a gradual adaptation which after the fourth passage was manifested by good reactions. Experiments with the pigeon virus after various passages on the fowl indicated a progressive increase in virulence for the fowl and, as shown by generalization and mortality of fowls inoculated intravenously, the pigeon virus apparently acquired "all the properties of fowl-pox virus."

Morcos (1931) reported that fowl-pox virus when passed thru pigeons, regardless of the number of serial passages, confers an immunity to fowls. Picard (1931, 1931A) reported that pox virus from turkeys, ducks, and pigeons, as well as formalized fowl-pox virus, was unsatisfactory for use in vaccination against fowl pox. Cutaneous vaccination with turkey and pigeon virus gave a strong local as well as a general reaction but an incomplete immunity. An efficient vaccine was at last obtained by passing the fowl virus thru pigeons. After 54 passages, over a period of two years, on the plucked breasts of pigeons, the original highly virulent virus had become attenuated to a lower but fixed or constant degree of virulence for fowls. According to the California Experiment Station (1933) the value of pigeon-pox virus as a vaccine in fowls was not increased by chicken passage. Hartwigk (1933) made comparative tests on various vaccine preparations and found that fowl-pox virus passed thru pigeons gave immunity in 100 percent of the chickens and pigeon virus in about 50 percent.

Doralp (1936) obtained pox virus from a severely affected turkey and "passaged" it four times on pigeons during a 10-day period. He claimed that two intracutaneous inoculations (.2 to .3 cc. dose) with this agent during an interval of 7 to 10 days resulted in successful immunization of fowls. Lubbehusen (1937) reported upon four years' experimentation to produce a modified virus vaccine for fowl pox, which possessed the immunity effect of fowl-pox virus and the non-

depressant properties of pigeon-pox virus. Three series of passages of two pigeon-pox strains were made with a gradual adaptation of the pigeon virus to chickens and a corresponding decrease in virulence for pigeons. In one series the adaptation to chickens was complete after 30 serial passages; in the two subsequent series the adaptations were slower and still incomplete after 70 passages. The experimental data, as interpreted, would suggest that the fowl-passaged pigeon virus possesses advantages over both types of vaccine (unmodified chicken virus and pigeon virus) now in use.

The virus known as antidiphtherin, prepared and introduced by De Blicck and Van Heelsbergen (1925), has been distributed and used quite extensively. Van Heelsbergen (1925) described "antidiphtherin" as:

" . . . a fully living vaccination material which is attenuated neither physically nor chemically, which always causes a local pox eruption and never gives rise to generalization, which is constant for all these properties and which protects for a long time against the experimental as well as against the spontaneous infection.

"After having experimented for over three years we have prepared such a virus."

Van Heelsbergen also reported the vaccination of 200,000 fowls with antidiphtherin in the winter of 1924-25 without a single "accident." Good results were recorded, also, from vaccinating affected birds. Vaccination during August and September was advised. An active immunity was claimed to persist for one to two years and the observation was made that the number of eggs from fowls vaccinated during the laying season was not diminished.

Doyle (1926) concluded that antidiphtherin was not uniformly attenuated, being weak or dead or sufficiently active to give rise to secondary lesions. According to his observations, the scabs produced by antidiphtherin could contaminate the premises, and hence the vaccine should be used only in infected flocks. Hol (1927) claims to have had marked success with antidiphtherin. He vaccinated quite a number of one-month-old chickens, which he deemed the youngest age at which vaccination could be done safely. That systemic disturbances from vaccination were obtained with the antidiphtherin is indicated by reference to dangers from vaccination under certain conditions. Leynen (1927) reported that he had been practicing vaccination with antidiphtherin since 1922 and stated that his results confirmed those of De Blicck and Van Heelsbergen. In 1923 cases of pox actually due to the vaccination and ascribed to an excessive virulence were seen but this defect of the vaccine was believed to have been overcome, as determined by later results. Vaccination between June and August was recommended. While it is stated that the vaccine does not affect egg production, preproduc-

tion vaccination is recommended and a systemic reaction after vaccination is recognized. The importance of proper handling of the vaccine and proper technic for vaccination is stressed.

According to Bierbaum, Eberbeck, and Rasch (1929), antidiphtherin proved to be a mixture of fowl- and pigeon-pox viruses. Zwick (1930) stated that antidiphtherin, originally virulent, had been so attenuated that there was no danger to hens. Baumann (1926) was of the opinion that it was a pigeon virus. Glover (1931) concluded that "the vaccine of De Blicck and Van Heelsbergen is indistinguishable from pigeon virus. Both are of value in the production of an active immunity and are without danger when employed under suitable conditions." Later De Blicck and Van Heelsbergen reported that they had succeeded in standardizing their antidiphtherin so that it was always constant in composition and action, and that it neither caused generalization nor produced any injurious effect upon the vaccinated fowls. At that time antidiphtherin had been used with no untoward effects to vaccinate over one million fowls. Such vaccination afforded a degree of immunity which protected the fowls for at least one year.

Van Heelsbergen (1934) stated that antidiphtherin was not a pigeon-pox virus but fowl virus modified by passages thru another animal, the species of which was not given. De Blicck (1934) defined antidiphtherin as an original fowl-pox strain which thru pigeon passage was suitably modified for the fowl. Graham and Barger (1936) stated that antidiphtherin behaves like pigeon-pox virus.

Mixed Fowl- and Pigeon-Pox Virus

Bierbaum, Eberbeck, and Rasch (1929) claimed excellent results from the use of a mixture of pigeon- and fowl-strain viruses in vaccination against fowl pox in chickens; while Zwick, Seifried, and Schaaf (1928) recommended that double or mixed virus (fowl and pigeon) be employed for immunization of fowls. Rasch (1930) believed that the vaccine of Lahaye was a mixture of fowl and pigeon virus, the latter predominating. Leyhausen (1933), however, cited one example of a flock in which fowl pox, with considerable mortality and potential stunting of growth, occurred six to eight weeks after the use of a vaccine which, the producer said, consisted of pigeon virus to which a small quantity of fowl virus had been added. Komarov and Kligler (1936) reported that no advantage could be found in using a double or mixed vaccine or a double method (pigeon-pox virus followed in two weeks or later by fowl virus) of vaccination. However, it was suggested tentatively that late hatches of chickens be vaccinated with pigeon pox and two months later with fowl pox.

Vaccine Virus and Fowl-Pox Virus

The question of the relationship between the viruses of vaccinia and fowl pox has attracted the attention of numerous investigators. Immunological and cytological studies have led to highly divergent views, but in spite of the opinions of Toyoda (1924), Pandit (1927), Van Nederveen (1926), Matsumura (1934), and others, as expressed by Zwick (1930), that the viruses of the various animal poxes are varieties of an original pox, probably variola, present opinion largely holds that fowl-pox virus and vaccine virus differ distinctly in immunological and certain other characters. The original view of Jenner (1789) that the virus of vaccinia is a form of variola modified by cow passage in its virulence for man is now universally upheld. Van Heelsbergen (1920) reported that vaccine virus produced vesicles in fowls with a subsequent greater tendency to suppurate than did fowl-pox lesions. It is stated that an apparent local but not a general immunity to cow-pox virus after fowl-pox infection was demonstrated. Levaditi, Harvier, and Nicolau (1922) were unable to demonstrate any cross-immunity between vaccinia and fowl pox. Gwatkin (1925) was unable to induce lesions on the combs of fowls with vaccine virus; and the birds were later found to be susceptible to fowl-pox infection. Toyoda (1924), however, claimed to have passaged fowl-pox virus thru rabbits, and with this material to have protected a child, sheep, and fowl against cow pox. He also claimed that calf lymph gives protection against fowl pox in man. Ledingham (1924) and Lusena (1925) failed to show any cross-immunity between fowl pox and vaccinia. Loewenthal, Kadowaki, and Kondo (1925) found that fowl passage of vaccine virus did not increase its virulence for fowls, nor did the lesions show any tendency to assume the appearance of fowl pox, as reported by Van Heelsbergen. Reciprocal immunity was not demonstrated between cow- and fowl-pox virus. Andervont (1926, 1926A) reported that altho the fowl is susceptible to vaccinia, an immunity is produced only to vaccinia and not to fowl pox. Fowl-pox infection failed to induce resistance to vaccine virus, and hence Andervont concluded that these two viruses are not identical nor even closely related. Blanc and Melanidi (1926) state that the two viruses are different and do not manifest a reciprocal immunity in fowls. Pandit (1927) claimed to have succeeded in transforming the virus of fowl pox to that of cow pox by successive passages on calves and monkeys. Doyle and Minett (1927) found that vaccine virus applied to the scarified combs of fowls produced well-developed lesions by the fifth day. These consisted of small, white, discrete pustules easily distinguishable from the dry, yellow, or brown granulating lesions of fowl pox. They were unable to infect pigeons and ducks with vaccine virus. Similar negative results, as reported by Van Heelsbergen (1920), were obtained from

intravenous injection of vaccine virus into fowls. Continued fowl passage of the vaccine virus was accomplished by Doyle and Minett (1927) thru five series only with difficulty, the reactions becoming progressively less pronounced until the sixth transfer was negative. Reciprocal immunity was not demonstrated between the two viruses in fowls.

Lahaye (1928) reported that the pigeon was quite refractory to vaccinia, and that this virus did not induce immunity to pox in this species. The fowl was found more susceptible to vaccinia but did not develop immunity from infection. He concluded that vaccinia and contagious epithelioma are entirely distinct entities. Findlay (1928) failed in attempts both to convert strains of vaccinia to fowl-pox virus by passage upon the fowl, and to induce in chickens inoculated with vaccinia an immunity such as that resulting from inoculation with any one of three strains of fowl virus. He concluded that no evidence was obtained to show any relation between the virus of vaccinia and the three strains of fowl-pox virus investigated.

Findlay also confirmed the observation of Andervont (1926, 1927) regarding the greater susceptibility of young chickens (one week old or less), by finding that vaccinia in the skin or comb of adults produced only small distinct papules, but in the skin of the young chick induced large yellowish growths indistinguishable from fowl-pox lesions. The histological changes of the skin were observed to be identical with both viruses. Zwick (1930) states that vaccination of chickens with vaccinia against fowl pox gave negative results. However, reference is made to immunizing hens by using a mixture of fowl pox and vaccinia virus previously passed in cattle. Ludford (1928) observed that the virus bodies produced by vaccine virus in the epidermal cells of the skin and cornea of the chick were the same as those induced by fowl-pox virus; that their structure was the same; that they originated in the same manner and underwent the same development. Furthermore vaccine virus failed to produce virus inclusions in the skin of the pigeon, whereas a bipathogenic virus of fowl origin induced this specific change.

Doyle (1930), in discussing the relationship of fowl pox and vaccinia, commented upon the negative results of cross-immunity tests. Woodruff (1930) offers additional evidence of a histological nature that the viruses of vaccinia and fowl pox differ greatly. Fortner (1931) concluded that it is impossible to protect chickens against fowl pox by variola-vaccine inoculation. Neither does fowl-pox infection increase resistance to vaccinia. Brunett (1933) did not obtain protection against fowl pox in chickens after infection with vaccinia. Matsumura (1934) concluded from his studies that variola, vaccinia, and fowl-pox virus are merely types of the same virus, as previously claimed by Toyoda (1924). Vaccinia virus was apparently altered

by fowl passages not only in the nature of the lesion which was produced but in immunological specificity for the original virus. After the second fowl passage, the virulence of the strains employed was considerably modified, and after the third to fourth passage the lesions induced were said to be indistinguishable from fowl pox. Matsumura (1934) stated that the Korean and American strains of fowl-pox virus employed were not so readily adaptable to rabbits as was the Japanese strain.

Irons (1934) failed to demonstrate cross-immunity of vaccinia and fowl pox on chickens. He observed that the strain of vaccine virus employed was pathogenic for the chicken but not for the pigeon and sparrow. The lesions produced by vaccinia were not altered appreciably by repeated chicken passage, while the lesions of vaccinia and bird pox on the chickens were considered to be readily distinguishable by staining reaction of the inclusion bodies. Graham and Barger (1936A) vaccinated chicks varying in age from 7 days to 17 weeks, and altho obtaining takes in 45 percent of the vaccinated birds, concluded that no resistance was induced to subsequent artificial exposure to fowl pox. Findlay (1928) pointed out that the variable results reported would not, in view of observed differences in various strains of fowl-pox virus, exclude the possibility "that certain strains of fowl-pox virus may have some affinity with the virus of vaccinia." Nevertheless, it was suggested that the results by Toyoda, Pandit, and Van Nederveen may be explained by the possibility that the hens from which the virus was originally obtained were infected not with fowl pox but with vaccinia.

Additional evidence beyond criticisms as to methods and means of test and maintenance of purity of the virus or viruses employed would seem desirable, altho it would appear that few, if any, strains of fowl-pox virus have a significant affinity for various mammals.

Immunization of Pigeons

In the earlier references pertaining to the susceptibility and reaction of the pigeon to pox the sources of the virus—that is, whether pigeon or fowl—are not indicated. Nevertheless, the more recent reports dealing with immunization generally specify the immediate source of the strain of virus, altho in most of these a determination of the bi-pathogenic and mixed properties of the virus employed has not been made. Loewenthal (1906) claims a partial or complete immunity following intraperitoneal injection of the virus; while, according to Goodpasture (1928), Lipschutz found that subcutaneous injection of 1 cc. of a fairly thick suspension of virus immunized some but not all pigeons. Findlay (1928) reported that after "vaccination" with a bi-pathogenic virus of pigeon origin some slight immunity was still present 150 days later; two monopathogenic fowl strains which did

not produce significant lesions gave only slight protection after 10 to 20 days. Rasch (1930) reported that of six pigeons inoculated cutaneously in the skin of the leg with the Lahaye vaccine (pigeon strain according to Lahaye), all showed well-marked lesions which persisted for three to five weeks. When inoculated later (time not given) with virulent pigeon virus all of these birds, together with two controls, developed extensive pox. Fowls were also inoculated with this vaccine and inoculation reactions were produced, but when tested 37 days later with virulent fowl-pox virus all became infected. Rasch stated the belief that the Lahaye vaccine was a mixture of pigeon- and fowl-pox viruses, the former predominating.

Stafseth (1931A) described an outbreak of pox in pigeons in Michigan and recommended vaccination of all pigeons used for show purposes and those which may be exposed to birds returning from shows.

Glover (1931) stated that chickens were not appreciably protected with heated or chemically treated fowl-pox virus, as were pigeons with similarly treated pigeon virus. Morcos (1931) reported that pigeon virus treated with .2-percent formalin and kept at room temperature for two days protects both pigeons and fowls against the homologous virus; tests for immunity to the heterologous virus were not made. Pyle (1932) stated that the vaccine made from pigeon virus is efficient as an agent in protecting pigeons against pox and diphtheria, or "canker"; and that pigeons as young as six weeks may be vaccinated with safety. The vaccine is rubbed into three or four open feather follicles on the thigh with a stiff brush. In 1937 Pyle observed that "experience has shown that pigeon-pox vaccine must be used rather cautiously on pigeons."

The Illinois Station Report for 1934 (Graham and Barger, 1935) records that both the stick and feather-follicle methods of application of pigeon virus (Illinois strain) to pigeons produced severe local reactions. In 58 pigeons treated with fowl virus by the feather-follicle method, 90 percent showed a mild folliculitis after one week. No immunity to the homologous virus could be demonstrated after one, two, and three months. Irons (1934) stated that pigeons inoculated with a strain of fowl-pox virus latently bipathogenic and adapted to the pigeon by serial passage showed as marked protection as those inoculated with the pigeon virus. He observed that with pigeon passage there was an increase in the virulence of the pigeon virus and a decrease for the chicken virus, the latter, however, being restored by serial passage in chickens. Basset (1935) reported that it is necessary to use homologous virus to induce a strong degree of resistance in the pigeon. Of five young pigeons inoculated intramuscularly with pigeon-strain virus, three developed complete protection and two a partial protection against subsequent artificial exposure.

Wittmark (1937) found that pigeon virus treated with bile, phenol, or formol produced quantitative attenuation but no qualitative change. Such vaccines sometimes caused intense vaccination reaction or no response whatever. Cutaneous infection with the virulent virus confers absolute immunity in pigeons; but many of the pigeons, particularly those under three months of age, may die as a result of vaccination infection.

Factors Bearing on Vaccine-Host Relationship

Measures used with the object of securing satisfactory immunization against pox in various species of birds have apparently fallen short of the goal, as judged by reports of different investigators. These irregularities seem to be largely related to factors of biological variation. Even where suitable modification of virulence of a certain virus has been effected and "fixed" in an apparently narrow range, with retention of desirable antigenic characters, the fluctuations in susceptibility of the host occasionally may vary considerably beyond the limits of safety and even lead to "accidents" or undesirable reactions.

Among factors having an important bearing upon the vaccine-host relationship are: (*a*) method of applying the viruses and vaccines, including dosage; (*b*) age of the bird as related to post-vaccination reactions, as well as inadequate immunity responses; (*c*) degree and duration of immunity as influenced by kind of vaccine or virus; and (*d*) criteria for interpreting the results from vaccination with various viruses and vaccines.

Methods of application. The "vaccines" of early workers (skin or mucous-membrane lesion material subjected to physical or chemical treatments, or both) were applied largely by injection into the subcutaneous tissues. Loewenthal (1906) claimed that cutaneous immunity was produced after intraperitoneal injections of virus, presumably that of fowl pox. Lipschutz (1908) claimed that the intravenous inoculation of virus suspensions immunized some but not all pigeons. Mantefel (1910) recorded successful results from injection of vaccine intravenously or subcutaneously. Van Heelsbergen (1920) reported that three intravenous injections of virus suspensions (apparently of pigeon origin) immunized fowls. Beach (1920A) reported that immunity was obtainable by intracutaneous injection of both heated and unheated pox material. Panisset and Verge (1923) introduced vaccine intradermally and reported successful results in both hens and pigeons.

With the introduction of virus vaccination, application to the skin via the methods of scarification, of open feather-follicle instillation, or a combination of the two, and by skin puncture (stick or scab), as employed by various workers already mentioned, came into general usage. The scarification and feather-follicle methods seem to lend

themselves particularly well where relatively large exposures or inoculations of the cutaneum seem desirable, as with the use of unmodified pigeon virus or certain biologically modified fowl or pigeon viruses. The stick method seems better adapted for use with highly virulent homologous virus where only a very limited exposure or a small dosage of inoculum is desired. Basset (1924, 1928, 1935), in advocating intramuscular inoculation of unmodified homologous virus, emphasized proper gradation of dosages to avoid vaccination sequelae. He contended that the cutaneous and subcutaneous routes, whereby the more susceptible tissues were exposed primarily or more directly to the virus, favored the occurrence of subsequent difficulty. Beach (1927) encountered undesirable reactions following subcutaneous application of virulent fowl virus, not unlikely associated with the excessive dosage of the virus injected and low vitality of birds.

In introducing the stick method Johnson (1929) specified its advantages over the feather-follicle method in vaccinating birds in molt, and stated that it obviously would allow more uniform and rapid vaccination in other birds regardless of whether or not they showed follicles satisfactory for vaccination by the older method. In 1930 Johnson stated that "no difference in the degree of immunity has been apparent between one stick vaccination per fowl followed by a mild lesion unaccompanied by scab formation at the point of take and more extensive vaccination reactions followed by scab formation." Martin (1933) and Johnson (1934), from experience in the field, listed a number of advantages of the stick over the feather-follicle method.

Komarov and Kligler (1936) observed that with phenolized fowl-pox vaccine the size and intensity of the primary take have an important bearing on the occurrence of secondary lesions and confirm Johnson's observations (1929) that the stick method reduces to a great extent the incidence of secondary lesions. Van Heelsbergen (1925) stated that intradermal injection as practiced by Verge and Panisset, even tho only small quantities were injected, did not prevent generalization. Cutaneous vaccination by scarification was found by this author and De Blicke to offer desirable advantages over other routes. Doyle and Minett (1927), after comparing the effect of the route of the introduction of fowl virus upon the subsequent severity of the reaction in fowls, found that inoculation of the mucous membrane was almost invariably followed by generalization and death, while such results were much less common after inoculation of the plucked skin. Subcutaneous inoculation appeared to exert a milder effect than the intravenous route, while intramuscular introduction of the virus was followed by a similar but less severe reaction than that from subcutaneous inoculation. The work of Findlay (1928) indicates that the route of injection influences the degree and extent of ultimate involvement of the epithelium, particularly in so far as the participation of

the reticulo-endothelial system is concerned, in clearing the system of virus.

Other observations would also suggest that the immediate as well as ultimate fate of the virus introduced into the body determines whether or not immunity is produced; for, if the infective agent is phagocytized and removed without localization and "colonization" in epithelial tissue rendered susceptible to the virus as a result of recent injury, a demonstrable immunity is not induced. With full development and subsequent resolution of lesions, the escape of virus and its appearance or disappearance in the circulation have been repeatedly demonstrated. However, the development of detectable focal pocks is apparently not needed to stimulate immunity, according to conclusions of Zwick (1930), who stated that "the appearance of local phenomena is not necessary for the creation of immunity. Intravenous, subcutaneous, and intramuscular inoculation can all produce immunity." That antiviral immunity, altho it may represent "isolation" of the susceptible tissue or cells (in this instance, primarily the epithelium), must originate chiefly in the reticulo-endothelial system is not inconsistent with present opinion in immunology and would seem to clarify the preceding observation. Of interest in this connection is the observation of Beach (1927) that following subcutaneous injection of virulent fowl-pox lesion material, "the percentage of fowls that became completely immunized will be greater among those that have developed vaccination-point lesions (of the skin) than among those that have not."

It is commonly recognized that great variation in general and specific resistance among vaccination subjects is a serious hazard in the use of vaccines. Nevertheless, uniform dosage and proper impregnation of a titrated dose of virus or vaccine would seem of primary importance. In application of the stick method of vaccination with fowl virus, Johnson (1934) suggested that certain newer instruments devised for the purpose may complicate the procedure and defeat the simplicity and efficiency of the earlier methods.

Regarding dosage and virulence, Beach (1927) concluded, in connection with subcutaneous injection of fowl-pox virus, that "the immunizing value of vaccine has been shown to depend upon and to vary according to the amount and virulence of the virus it contains. No method has yet been devised, however, by which less than marked differences between the virus content of vaccines can be detected." In 1936 Lubbehusen, Beach, and Busic reported that in using regular commercial fowl-pox vaccines, the regular concentration of these products (1-percent virus suspensions) was suitable for chicks, and that a special chick vaccine of greater concentration was undesirable. A special inoculating needle for stick vaccination was devised and recommended. Later Lubbehusen and Beach (1937) tested virus con-

centrations of 1, 2, and 3 percent and found that the 3-percent virus vaccine tended to cause more extensive and persistent takes, and a greater but temporary retardation of growth, than vaccines of lower virus concentrations. With potent vaccines, however, there appeared to be no differences in the certainty with which these virus concentrations produced takes. Broerman and Edgington (1928), referring to the quantity or dosage of fowl virus administered to chickens, as determined by the number of feathers plucked, observed a greater percentage of immunity in fowls treated with pigeon virus by a single feather-follicle inoculation than by the single-stick method. The criticisms of Doyle (1933 and 1935) of the work of Michael (1932) and of Delaplane and Stuart (1933) with pigeon-pox vaccination in fowls is apparently directed toward the factor of inadequate dosage presumably associated with the unsatisfactory methods of preparation and application of the vaccine. Johnson (1930) claimed satisfactory takes with both the one-stick method and the scarification method of vaccinating fowls with pigeon-pox virus, but did not record any determinations for subsequent immunity to fowl pox.

Analysis of the reports herein cited reveals that the use of unmodified homologous pox virus for vaccination represents an effort to induce an active immunity without inducing undesirable disturbances. Success of the method apparently depends on securing, under highly variable conditions, the proper dose of the infective agent, which obviously may not be the same for all fowls. The hazards of such a procedure have been suggested in practice. Hence it is recognized that safer prophylaxis against fowl pox may lie in the use of a virus with negligible disease-producing characters (heterologous virus) or a homologous virus modified in virulency but not in antigenicity by passage adaptation to a different species of host.

Influence of age upon immunity. Altho birds of all ages are known to be susceptible to fowl pox, it appears that very young chickens are more susceptible than older growing birds or mature fowls and, furthermore, that other infections and management methods may alter or lower vitality and thus influence susceptibility to pox as well as the immunity induced by vaccination. Findlay (1928) found chickens one day to one week old much more uniformly susceptible than mature birds, not only to fowl pox but also to vaccinia virus; and it is highly probable that this greater susceptibility is reflected in the more frequent occurrence of serious post-vaccination sequellae in chicks vaccinated when very young. Irons (1934) reported that baby chicks proved much more susceptible to pigeon-pox virus than did adult chickens, the former showing lesions more nearly like mild fowl-pox lesions in which Bollinger bodies could be readily identified. These bodies were absent or difficult to find in pigeon-virus lesions in adult fowl. From field observations Johnson (1929) believed it inad-

visible to vaccinate fowls under three months of age because younger birds appeared to be more severely affected. Later Johnson (1934) reported that day-old poults and 2-day-old chicks vaccinated with one needle stick on the second day after hatching showed slight but distinct protection against inoculation about eight and one-half months later. In vaccinating 1- to 8-day-old chicks Danks (1931) encountered generalization of inoculation pox infection as well as stunted growth and considerable mortality, and hence concluded that it is seldom advisable to vaccinate chicks less than one month old. The immunity induced was found inadequate to protect against artificial exposure to fowl virus two months later. Dunn and Sherwood (1933) reported immunization of day-old chicks and poults with dilute fowl-pox virus applied by scarification without causing an apparent constitutional disturbance. However, the degree and duration of immunity induced by such vaccination were not determined. Because of high mortality following vaccination of chicks less than 4 weeks of age, Bice (1933) undertook to prepare a vaccine which would be suitable for use in chicks 2 weeks of age. Lubbehusen and Ehlers (1932) reported evidence of post-vaccination systemic reaction manifested by interruption of growth gains in normal birds vaccinated when older than 120 days. This reaction, however, was not observed in birds vaccinated at 30 to 90 days of age. Birds vaccinated when less than one month old were not included in the observations.

Devolt, Moulthrop, and Davis (1936) vaccinated 73 12-day-old chicks against fowl pox by the stick method. Severe systemic reactions developed during the third and fourth weeks after vaccination. In testing the degree and duration of immunity following fowl-pox virus vaccination of day-old chicks, Lubbehusen and coworkers (1936) reported that the data, altho still incomplete, indicated that the severity and duration of the take, as determined by growth gains and mortality associated with vaccination, were less severe in chicks vaccinated at 3 weeks than in those treated at one week. In 8-week-old chicks the systemic reaction was apparently absent, yet chicks showing a satisfactory take after vaccination at one day were reported resistant to contact exposure as long as eighteen months later. In conclusion these authors called attention to the possibility that reaction and mortality immediately attributable to vaccination may tend to eliminate at an earlier age birds of low vigor and vitality and hence contribute to decreased losses during the later growing or adult periods.

Immunity influenced by type of virus. An important aspect of the problem of immunization against pox in chickens and pigeons concerns the fact that the majority of strains of pox virus derived from pigeons are infective for chickens (bipathogenic). On the other hand, most strains presumed to be of fowl origin appear to be infective for chickens only (monopathogenic). The significance of this

circumstance is apparent since it is highly probable that active immunity to avian poxes is induced only on introduction of active virus and then only if infection, even tho inappreciable, occurs.

As early as 1873 Bollinger recognized that pigeons are generally resistant to infection with fowl-pox virus. Doyle and Minett (1927) reported that after previous failures they were able to adapt a strain of fowl virus to the pigeon by frequent serial skin passage. On the second series of pigeons the feather follicles were slightly swollen. There was a progressive increase in the swellings with succeeding passages, and from the fifth series on the lesions were well defined and similar to those in fowls inoculated by the same route. These authors cited the negative results of Jowett (1909) and Carnwarth (1907-08) in attempts to infect pigeons with fowl-pox virus. Gallio-Valerio (1925) also reported transmission of fowl virus to pigeons. Zwick, Seifried, and Schaaf (1928) and Bierbaum, Eberbeck, Rasch, and Kayser (1931) recognized monopathogenic and bipathogenic strains of fowl and pigeon viruses, of which only the latter were cross-infective. Brunett (1933) stated that fowl-pox virus could not be propagated readily in pigeons, while Irons (1934) employed five strains of pigeon virus in his studies and found all infective for chickens. Bierbaum (1935A) found a strain of pigeon virus that was not infective for chickens. This strain may have represented an original sparrow or other wild bird strain which, according to the studies of Irons (1934) and others, may possess greater potential infectivity for pigeons than for fowl.

References to cross-infectivity or pathogenicity of pox viruses of poultry other than chickens, and of pigeons as well as various wild birds, suggest that among these strains marked variations may prevail. As a source of modified virus potentially suitable for vaccination in chickens, turkeys, or pigeons, the pox derived from or adapted to other species of birds has been given little attention. Ward and Gallagher (1920) stated that pox occurs naturally among geese, ducks, and guinea fowl, and that pheasants and various wild birds are also susceptible. According to Goodpasture (1928), pox occurs in pheasants and hawks. Gallagher (1916) described an outbreak of pox in quail, transmissible to fowl. Te Hennepe (1926) reported pox in 17 of 268 ducks received for diagnosis. Doyle and Minett (1927), Findlay (1928), and Irons (1934) were unable to infect ducks with fowl pox. One seagull was tested by Doyle and Minett and proved refractory also. Findlay (1928) identified lesions of pox in partridges but could not infect chickens with the lesion material. Tietz (1932) concluded that ducks could not be infected with fowl or pigeon viruses; that turkeys and guinea fowl are susceptible only to fowl-pox virus, not to pigeon virus, but that the crow is susceptible to both; furthermore, that monopathogenic fowl and pigeon virus is nonpathogenic for

canaries, finches, siskins, sparrows, starlings, and thrushes. Irons (1934) likewise obtained negative results in attempting to infect pigeons with a turkey virus and also failed to infect crows, hawks, owls, ducks, guinea fowls, starlings, and several other species with fowl and pigeon strains. One strain of pox from a wild pigeon proved infectious for the English sparrow and certain related species.

Brunett (1934) found the turkey susceptible to turkey, fowl, and pigeon virus, the latter producing a more severe focal reaction than in the chicken but without establishing demonstrable subsequent immunity. The general opinion exists that turkey strains of pox virus are very similar, if not identical, in infectivity or pathogenicity to those of fowl origin. Coronel (1934) used for vaccination of chickens a strain of turkey-pox virus which prior to aging at 0 to 5° C. was apparently quite virulent for chickens. However, Irons (1934) found that a turkey strain of virus did not produce lesions typical of fowl pox in chickens until after several serial passages on chicks. A turkey strain which apparently was not infective for chickens came to the attention of the Illinois Experiment Station during 1937.

Present knowledge of pox in birds suggests that pathogenic or infective characters are apparently requisite for the manifestation of antigenic function and consequent stimulation of the immune state. However, marked differences in antigenic efficiency have been demonstrated among various strains of pox viruses from birds, particularly immunization experiments with cross-species. The fact that infection, with marked focal reaction, may occur with pigeon virus in the turkey without engendering a demonstrable immunity, as reported by Brunett (1934), suggests that similar results may be expected with other viruses employed in heterologous hosts.

A number of workers, including Doyle (1930), Stafseth (1931), Brunett (1933), Delaplane and Stuart (1933), Kligler, Komarov, and Fiat (1933), Edgington (1934), Lubbehusen and Ehlers (1934), and Graham and Barger (1935), have observed that the immunity induced by vaccination of chickens with homologous virus is more profound and durable in nature than that procured with heterologous virus or vaccine. Basset (1935) cites experiments to show that vaccination with homologous virus is necessary to produce a solid and lasting immunity in pigeons and chickens to the homologous virus. With reference to the use of homologous and heterologous virus on pigeons, the results of Findlay (1928), Pyle (1932), Irons (1934), and Graham and Barger (1935), already cited, support the greater efficiency of homologous virus for immunizing this species. Irons (1934) pointed out that bipathogenic fowl-pox virus adapted to the pigeon produced an immunity equally as strong as that obtained with pigeon virus.

Doyle and Minett (1927) were unable to show any immunological distinction, in fowl-protection tests, among eleven strains of fowl-pox

virus used in the course of their work. Lahaye (1929-30), in a study of pox in fowls, turkeys, and guinea fowls, did not find any immunological differences. Findlay (1928) reported two strains of fowl origin antigenically alike. A third bipathogenic strain apparently of pigeon origin manifested evidence of bi-antigenic types. Immune serum prepared with this strain neutralized both components, while antiserum for monopathogenic fowl strains neutralized only the factor infective for chickens. Bierbaum and Kayser (1933) showed that two strains of pigeon virus were identical by cross-immunity tests and did not show evidence of plurality. Finkelstein (1934) studied the immunological relations of fowl and pigeon pox by means of macroscopic agglutination tests. Hyperimmune sera were prepared from fowls treated with fowl- and pigeon-pox viruses over a long period. Fowl-pox antiserum agglutinated pigeon as well as fowl-pox elementary bodies, but to a lower titre than the homologous corpuscle suspensions. On the other hand, pigeon-pox antisera were not found to agglutinate fowl-pox bodies.

In general, passage thru heterologous species appears to effect, sooner or later, alterations in the virulence of the virus. Whether or not attenuation in virulence by such a procedure may cause also a loss or modification of antigenic quality and efficiency is not fully determined. Marx and Sticker (1903) observed that a single passage of pigeon virus on the chicken frequently rendered it avirulent for the pigeon. Irons (1934) reported that after a single chicken passage the virus of pigeon pox was greatly attenuated but further passages failed, with one possible exception, to destroy the infectivity for the pigeon. The virulence of pigeon virus for fowls was greatly enhanced by chicken passages; whereas the bipathogenic fowl virus was temporarily attenuated for the chicken when passaged in pigeons but was increased in virulence for the pigeon. However, Findlay (1928), as well as Bierbaum, Eberbeck, and Kayser (1931), found only a slight and temporary reduction in virulence of pigeon virus for pigeons after numerous passages on chickens. Lahaye (1927) reported that passage of pigeon virus on fowls did not attenuate it for the pigeon. Saito (1926) recorded a loss of virulence of pigeon virus for chickens with repeated chicken passage, while this property remained constant for the pigeon. Serial passage of fowl virus on the pigeon was said to render it more virulent for the pigeon and less virulent for the fowl. Doyle and Minett (1927) observed that passage of pigeon virus on chickens thru nine series caused the production of lesions identical with those induced by strains of fowl virus and effected a change which prevented ready readaptation to the pigeon.

Lubbehusen (1937) reported gradual adaptation of pigeon strains to chickens, with a corresponding decrease in virulence for pigeons and with immunological advantages over unmodified fowl or pigeon

virus. Danks (1931), California Experiment Station (1933), and Michael (1932), working with original pigeon virus, reported little or no immunity in contrast with the lasting immunity to natural or artificial infection of a year or more, as reported by Lahaye (1928), Cominotti and Pagnini (1931), and E. P. Johnson (1931, 1932). The fact that a partial but definite immunity to artificial as well as severe natural exposure to fowl pox may be obtained for periods of two to six months and occasionally longer is apparent from the findings of various other workers, including Doyle (1930), Glover (1931), Broerman and Edgington (1932), Brunett (1933), Delaplane and Stuart (1933), Graham and Barger (1935), Bierbaum (1935), Basset (1935), and Komarov and Kligler (1936). Altho Basset states that complete protection may be obtained by inoculation of a large area of the chicken skin with pigeon virus (practically not feasible), he does not state the period of duration of such an absolute immunity.

In vaccination of chickens with homologous virus, even a mild take apparently induces a strong and durable immunity (Johnson, 1930). This author has recorded a distinct, altho incomplete, degree of protection against artificial exposure as long as 967 days following vaccination. Johnson (1934), however, expresses the conclusion, apparently consistent with findings and observations of numerous investigators on this point, that "chickens seldom if ever maintain complete immunity for extended periods, following vaccination with fowl virus." The results obtained with pigeon and fowl viruses adapted to heterologous species suggest that the eventuality of continued serial passage will be the development of characters closely resembling or typifying the homologous virus. The observations of De Blicck and Van Heelsbergen on antidiphtherin, which is said to represent a fowl virus adapted to pigeons, emphasize its similarity to pigeon-pox virus; that is, in so far as gross appearance of the lesion and degree and duration of immunity are concerned. Picard (1931 and 1931A) reported similar changes resulting in fowl virus after fifty-four passages in pigeons, while Lubbehusen's report (1937) on adaptation of pigeon virus to fowls indicates that protracted serial passage is essential for modification and fixation of these characters.

The literature on vaccination of pigeons lends support to the opinion that, in general, homologous virus vaccination induces a substantial immunity of considerable duration. Findlay (1928) found that exposure to monopathogenic virus obtained from fowls failed to provoke more than an atypical transient reaction and the protection, if any, may be only nonspecific in nature and apparently insignificant. On the other hand, pigeon-virus (bipathogenic) vaccination resulted in a slight but demonstrable immunity 150 days later. Basset (1935) emphasized the necessity of homologous virus for vaccination of pigeons for production of strong resistance. According to Irons (1934),

pigeons inoculated with a bipathogenic fowl strain of virus adapted to the pigeon by limited passages gave as marked protection as did the pigeon virus.

Criteria for interpreting results. The reported results from fowl-pox vaccination are often difficult or impossible to interpret accurately because of failure to recognize or employ satisfactory criteria. In many cases, the effect of vaccination on subsequent growth and vitality has been entirely or largely overlooked, altho the more apparent factor of mortality associated with vaccination has been generally appreciated. The common and erroneous deduction that complete immunity persists for long periods also has led to much confusion.

The importance and significance of determining the effect of vaccination of chickens of various ages upon subsequent weight gains are illustrated particularly well in the reports of Lubbehusen and co-workers (1932, 1934, 1936, and 1937). The occurrence following vaccination of generalized systemic reactions, with or without secondary skin or mucous-membrane foci or pocks, has been encountered and recognized quite frequently as an undesirable sequel. The possibility of using the weight at maturity as a criterion for evaluating the influence of vaccination has been suggested by Johnson (1934) and others. The effects of fowl-pox vaccination on egg production have been noted by Johnson (1927 and 1930), Sawyer (1928 and 1929), Pyle (1928), and others.

Capability to produce a satisfactory take or reaction is considered a primary requisite for the virus material to be used for cutaneous pox vaccination. In birds of apparently normal susceptibility and previously unexposed to pox, the severity of the local lesion produced by vaccination would seem to be largely dependent on the extent and degree of exposure as well as on the virulence of the virus. Considerable variations associated with age and individual susceptibility are, of course, frequently encountered. According to Loewenthal (1906), cited by Goodpasture (1928), secondary pox infection in pigeons showed no diminution in intensity up to the time the scab resulting from primary infection fell off. According to Goodpasture (1928), Burnet in 1904 found that response to reinoculation within five days was equivalent to that in the control. The lesions of subsequent reinoculations gradually diminished in extent until those made on the twelfth day aborted completely. The lesions of reinoculation healed concomitantly with the primary lesions. Johnson (1930) referred to a quick reaction response to skin reinoculation of fowls which is frequently apparent in 24 hours, while Findlay (1928) found a certain degree of immunity in both chickens and pigeons 4 days after inoculation with homologous virus, and a complete immunity after 20 days. He states that Henseval and Convent (1910) noted a degree of immunity in 4 days following

cutaneous inoculation. More recent investigations on the mechanism of response and protection to foreign proteins, bacteria, and viruses appear to have clarified the role of fixation and inflammation in augmenting resistance and immunity. The findings of Opie (1924), Menkin (1931), Cannon *et al* (1929, 1930, 1932), and Kahn (1933, 1936) would suggest that reinoculation with pox virus during the interval of several days to about three weeks subsequent to original infection results in a variable degree of focal or regional fixation of the virus.

It is therefore conjectured that incorrect evaluations of immunity derived from vaccination have been made because of failure to recognize states of partial immunity by proper determination and interpretation of the response to reinoculation, particularly in the case of severe artificial exposure. In addition, other criteria among those mentioned must be established if the significance and value of any vaccine or virus or procedure are to be assessed properly.

PART II: ILLINOIS EXPERIMENTS

Fowl-Pox Immunization With Pigeon-Pox Virus

From the foregoing review of pox immunization in fowls it is evident that a diversity of opinion exists as to the efficacy of pigeon-pox virus as a vaccine for immunization against fowl pox in chickens. The results of studies at the Illinois Experiment Station over a period of five years (1932-1936) include observations on several thousand fowls vaccinated with pigeon-pox virus and subsequently exposed by artificial inoculation to fowl pox.

Source and preparation of virus. The pigeon virus employed was one obtained in 1930 from a natural outbreak of pigeon pox in an Illinois aviary (Fig. 1) and which was subsequently maintained by serial passage thru pigeons.

Various methods have been used by investigators for preparing pigeon-pox virus for vaccine. The following technic was employed at the Illinois Station: healthy mature pigeons were plucked over the ventral surface of the breast and a freshly prepared 1-percent aqueous suspension of powdered skin-lesion virus swabbed over the entire area without scarification (Fig. 2).

The time required for the development of typical scabs was found to vary within rather wide limits, being as long as 20 days in some cases and as short as 12 days in others, with an average of about 16 days. In 24 to 48 hours after inoculation the pigeons displayed a definite systemic reaction. The eyes were closed, the feathers were ruffled and often the bird showed slight tremor. A rather rapid loss of



FIG. 1.—NATURAL CASE OF PIGEON POX



FIG. 2.—APPLYING PIGEON-POX VIRUS TO FEATHER FOLLICLES ON BREAST OF PIGEON

flesh usually accompanied the development of lesions, and an impairment of appetite was noted. The inoculated follicles showed a definite swelling in 48 hours and the entire inoculated epiderm presented a diffuse, swollen, congested appearance. The reaction progressed until the 8th to the 10th day, while definite scab formation developed from the 12th to the 18th day (Fig. 3). At this stage the entire area was covered with a diffuse, hard, dry, brownish scab. Death often occurred

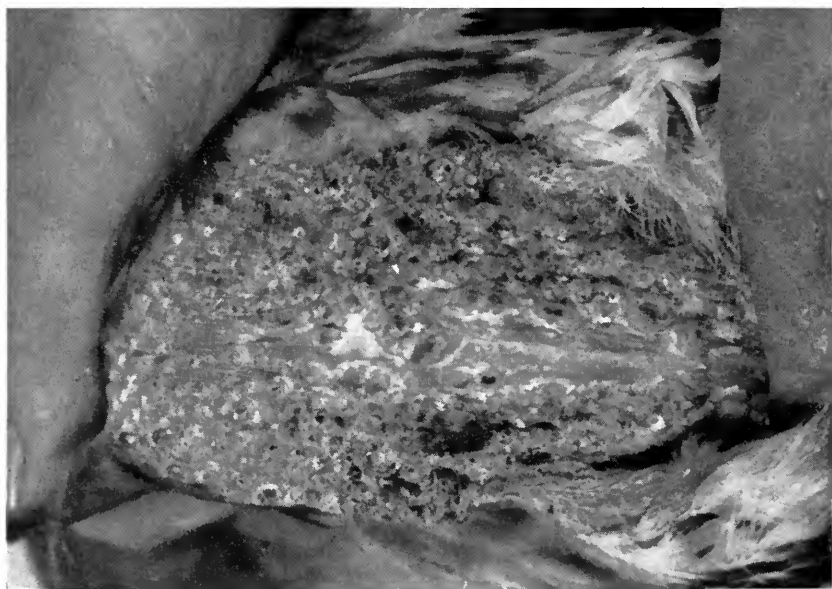


FIG. 3.—LESIONS OF PIGEON POX 20 DAYS AFTER INOCULATION WITH 1-PERCENT SKIN-LESION PIGEON-POX VIRUS

in the inoculated pigeon at about the 16th to the 18th day following application of the virus.

The scab mass on inoculated pigeons was removed just before death, altho death of the bird did not appear to alter the virulence of the virus, if the scabs were harvested within 12 to 15 hours. As a routine practice, inoculated pigeons were destroyed when in a moribund condition, usually at about the 16th day. During the period of scab development, a growth of new feathers occurred. These were plucked with a pair of thumb forceps before removal of the skin. Little difficulty was encountered in removing the skin and scabs en masse. The indurated skin was separated from the adjacent unaffected skin by means of a sharp scalpel. The entire piece of scabby indurated skin was then placed at room temperature in open Petri dishes and dried in

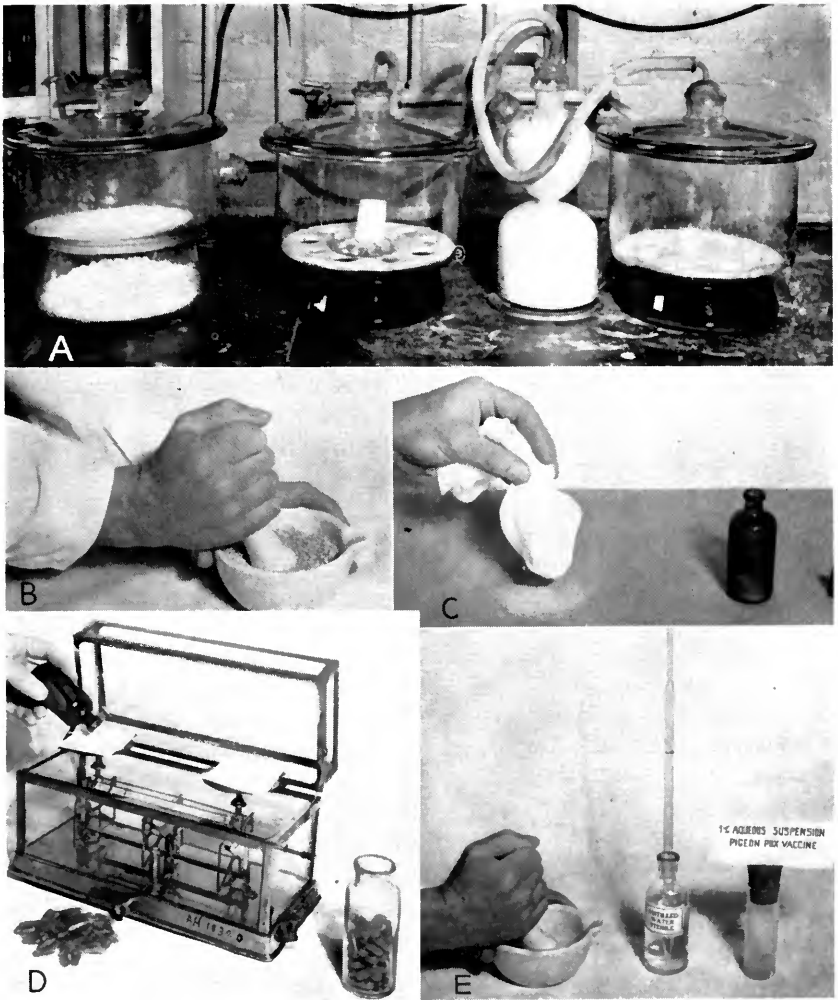


FIG. 4.—PREPARING SKIN-LESION PIGEON-POX VIRUS

(A) Drying virus, (B) grinding, (C) sifting thru clean gauze, (D) weighing in gelatin capsules, (E) mixing with distilled water.

a desiccator containing calcium chlorid and sulfuric acid (Fig. 4). After 4 days in the desiccator, the skin lesions were dehydrated and suitable for grinding. By means of scissors the dried skin was cut into small bits and then ground in a mortar to a fine powder. Coarse particles were removed by sifting the powder thru a single thickness of ordinary gauze onto clean or sterile filter paper. The powder was then placed in clean brown-glass bottles which were stoppered with

rubber corks, or in hard gelatin capsules and kept at ice-box temperature (10 to 15° C.). The powder constituted the stock vaccine used in the experiments described herein and, unless otherwise indicated, a freshly prepared 1-percent aqueous suspension of the powder was employed in experimental studies.

Application by feather-follicle method. Reports on the results of vaccinating chickens with pigeon-pox strains of virus indicate that, in general, more extensive takes and a somewhat greater degree of immunity are obtainable with the feather-follicle method than with scarification or puncture methods. The immunizing property of the Illinois strain of pigeon virus against fowl pox in chickens was determined by applying pigeon virus to the open feather follicles and later exposing the birds artificially to fowl-pox virus by a single skin puncture.

Procedure. In the experiments summarized in Table 1 a freshly prepared 1-percent aqueous suspension of pigeon virus was used for vaccination (with the exception of Lot 3). The site of vaccination was the upper lateral aspect of the tibial region of the leg, from which 10 to 20 feathers had been plucked. The virus was applied by rubbing the defeathered area with a cotton swab that had been dipped in the virus suspension. Observations for reactions were usually made at 7, 14, and 21 days after vaccination. Artificial exposure of vaccinated and control birds consisted, except where otherwise indicated, of swabbing a freshly prepared 1-percent aqueous suspension of fowl-pox virus over both sides of the scarified comb and over a plucked area on the leg opposite that used for vaccination. Natural exposure consisted of placing several fowls severely affected with fowl pox in the same pen with the vaccinated birds for a period of one month. Vaccinated birds and unvaccinated controls were simultaneously exposed to fowl pox by the same methods.

The control birds were of the same age, breed, and hatch as the vaccinated birds, and tho isolated from them received the same rations and care. The vaccinated and unvaccinated birds of each lot were kept separate, and in the majority of cases were isolated in small houses with concrete floors. Each house was surrounded by range (40 by 100 feet) to which the fowls had access at all times. During cold weather the smaller chickens were removed to houses equipped with heating units. When facilities permitted, chickens to be exposed to fowl pox were brought inside the laboratory and placed in special isolation pens. The unvaccinated control lot corresponding to each lot of vaccinated birds is designated by the lot number plus the letter C; that is, the controls of Lot 1 are identified as Lot 1C, those of Lot 2 as 2C, etc.

The development of mild lesions of fowl pox (folliculitis) at the point of inoculation without perceptible systemic reaction during the 14-day period following artificial exposure to fowl-pox virus was

TABLE 1.—RESULTS OF PIGEON-VIRUS VACCINATION AGAINST FOWL POX IN CHICKENS: FEATHER-FOLLICLE METHOD

Lot	Number of chickens	Breed ^a	Age	Vaccination reaction	Number exposed	Days from vaccination to exposure ^b	Results
1.....	22	RIR	<i>weeks</i> 18	Folliculitis	22	21	Partial immunity
1C.....	21	RIR	18	21	...	No immunity
2.....	81 ^c	RIR	3	Indefinite	5 5, 5, 5	14 21, 70, 98	No immunity Partial immunity No immunity
2C.....	81 ^c	RIR	3	5 25	112 ...	No immunity No immunity
3.....	18	BR	8	Folliculitis	9, 7	25, 51	Partial immunity
3C.....	6	BR	8	6	...	No immunity
4.....	21	WL	4	Folliculitis	10, 7	27, 53	Partial immunity
4C.....	6	WL	4	6	...	No immunity
5 ^d	20	BR	16	Folliculitis	17 ^d	21	No pox for 30 days
5C ^d	22	BR	16	22 ^d	...	36% developed pox
6.....	18	RIR	12	Folliculitis	17	35	Partial immunity
6C.....	2	RIR	12	2	...	No immunity
7.....	65	BR	4	Folliculitis	5 13	30 180	Partial immunity Partial immunity in 53%
7C.....	57	BR	6	51	...	No immunity
8.....	10	RIR	14	Folliculitis	10	60	Partial immunity
8C.....	3	RIR	14	3	...	No immunity
9.....	100	RIR	8	Folliculitis	24, 24, 24, 24	30, 60, 90, 120	Partial immunity
9C.....	12	RIR	8	12	...	No immunity
10.....	68	RIR	4	Folliculitis in 69%	30 15 7	70 120 150	Partial immunity Partial immunity in 60%, none in 26% Complete immunity in 42%, partial in 28%, none in 28% Severe pox
10C.....	67	RIR	4	59	...	No immunity
11.....	237	BR	6	Folliculitis in 97.5%	28, 25, 15, 15	30, 60, 90, 180	Partial immunity
11C.....	80	BR	6	80	...	No immunity
12.....	116	RIR	14	Folliculitis in 91.3%	30, 30, 15	30, 60, 90	Partial immunity
12C.....	30	RIR	14	30	...	No immunity
13.....	58	WL	5	Folliculitis in 27%	21, 31	27, 53	No immunity
13C.....	10	WL	5	10	...	No immunity

^aBR = Barred Rock; RIR = Rhode Island Red; WL = White Leghorn. ^bIn all the experiments involving control groups, a comparable number of unvaccinated birds were exposed at the same time as the vaccinated birds. ^cOnly 50 of 162 survived until exposure. ^dNatural exposure, all others artificial.

interpreted as an indication of a definite and significant partial immunity and in Table 1 is so recorded. These partial-immunity reactions were characterized by early benign pocks which receded quickly without systemic reaction, in contrast to marked and persistent pocks accompanied by systemic reactions following exposure to fowl-pox infection.

Results: Lots 1 and 1C, Rhode Island Reds 18 weeks of age (Table 1). All 22 birds in Lot 1 showed definite but mild takes, signifying

partial immunity. The 21 controls in Lot 1C proved uniformly susceptible on exposure to fowl pox.

Lots 2 and 2C, Rhode Island Reds 3 weeks of age. Each lot consisted of 81 birds at the beginning of the experiment. The occurrence of an outbreak of coccidiosis in Lot 2, with a high death rate, precludes accurate interpretation of the results. However, the birds exposed at 21 to 70 days following vaccination manifested definite evidence of a partial immunity. At 98 days, two of five vaccinated birds developed severe pox. All five of the vaccinated birds exposed at 112 days, as well as the controls, developed severe pox lesions, thus indicating that any significant resistance which may have been derived from vaccination had waned.

Lots 3 and 3C, Barred Rocks 8 weeks of age. The results in Lots 3 and 3C, comprising 18 and 6 birds respectively, indicate that a measurable but incomplete degree of resistance was present at about 25 and 51 days following vaccination with a .5-percent suspension of pigeon virus.

Lots 4 and 4C, White Leghorns 4 weeks of age. Only one of the 17 vaccinated birds in Lot 4 developed severe fowl pox following exposure, the latest exposure being made 53 days following vaccination. The six control birds in 4C manifested typical severe pox reactions.

Lots 5 and 5C, Barred Rocks 16 weeks of age. Lot 5 of 20 birds and Lot 5C of 22 birds were the only lots in this experiment which were subjected to natural exposure. Both lots were placed in a house in which pox-infected fowls had been kept continuously for the preceding three months without cleaning or disinfection during that time or following the removal of the affected fowl. In addition 10 hens showing severe fowl pox were introduced into the group to favor contact exposure. Failure of the vaccinated fowls in Lot 5 to contract infection for 30 days after exposure would seem to indicate at least a partial immunity to natural infection during 21 to 51 days following vaccination. The low percentage of infection in the controls cannot be definitely explained, but according to the experience of Doyle (1930) probably may be attributed in part to the roomy quarters, which did not favor close contact of test birds with affected birds.

Lots 6 and 6C, Rhode Island Reds 12 weeks of age. All 18 birds in Lot 6 developed satisfactory takes and, on exposure, a definite partial immunity was manifested. The two birds of control Lot 6C were found uniformly susceptible on exposure to fowl pox.

Lots 7 and 7C, Barred Rocks 4 and 6 weeks of age respectively. Lot 7 was originally comprised of 65 birds, but after heavy mortality resulting from coccidiosis and extremely cold weather, only 13 vaccinated birds survived for exposure at 180 days. However, only 6 of

the 13 vaccinated fowls developed a severe form of pox such as that occurring in the 51 unvaccinated controls.

Lots 8 and 8C, Rhode Island Reds 14 weeks of age. Sixty days following vaccination with pigeon virus the 10 vaccinated birds and 3 controls were exposed to fowl pox. All the vaccinated birds showed partial resistance, while the controls developed a severe form of pox.

Lots 9 and 9C, Rhode Island Reds 8 weeks of age. All vaccinated birds from the 100 originally constituting Lot 9, including those exposed at 120 days, appeared to possess a significant partial immunity to severe artificial fowl-pox exposure. The 12 birds of Lot 9C showed a natural susceptibility.

Lots 10 and 10C, Rhode Island Reds 4 weeks of age. In Lot 10, containing 68 birds, definite evidence of a waning resistance was manifested upon exposure at 120 days following vaccination. However, at 150 days the natural infection in 14 control birds of the original 67 in Lot 10C was about equal to that in the vaccinated group, thus invalidating any attempt at interpretation of the results.

Lots 11 and 11C, Barred Rocks 6 weeks of age. In Lot 11, comprising 237 birds at the outset, the plan to expose some birds vaccinated for a period longer than six months was disrupted by an outbreak of laryngotracheitis which decimated the remainder of the flock. Similar difficulty also interfered with further exposures in Lot 12, originally composed of 116 birds.

Lots 13 and 13C, White Leghorns 5 weeks of age. To determine whether or not aging of the powdered pigeon-pox virus decreased its immunizing value, the 58 birds in Lot 13 were treated with virus harvested 14 months previously and stored in the ice box at 12° to 15° C. Since severe lesions and systemic reactions developed in practically all the vaccinated birds as well as in the control group, it appeared that the period of aging under the conditions prevailing resulted in partial or complete loss of infective or antigenic activity of this strain of virus.

Preliminary experiment. In a preliminary experiment involving 24 Barred Rock chicks treated at one month of age (not included in Table 1), it was found that two methods of effecting uniform aqueous suspension of the powdered virus were equally effective, in so far as the percentage of vaccination takes and the immunity to fowl pox 28 days later were concerned. One method of effecting suspension consisted of adding the vehicle slowly to the powdered virus material in a glazed mortar while stirring the mixture continuously with a glazed pestle. In the other method the suspending fluid was added to the powdered virus in a vial; then the vial was stoppered and shaken vigorously for several minutes until uniform suspension was accomplished.

FIG. 5
PROTECTIVE CHARACTER OF PIGEON-POX VIRUS AGAINST
FOWL POX IN CHICKENS

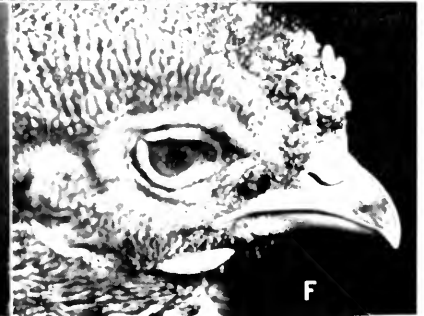
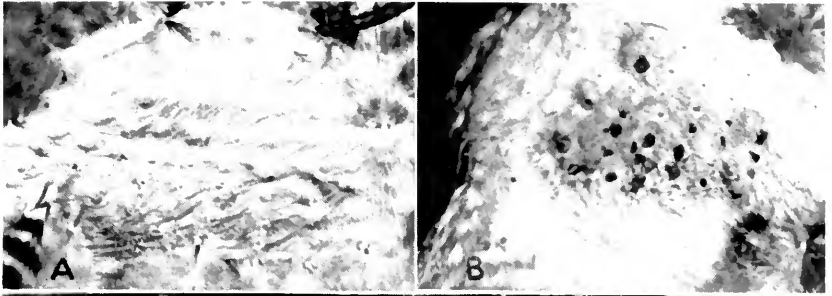
(A) Receding lesions on thigh of vaccinated fowl two weeks following exposure to a 1-percent aqueous suspension of fowl-pox virus, feather-follicle method. Six months before exposure this bird had been inoculated with a 1-percent aqueous suspension of skin-lesion pigeon-pox virus via feather-follicle method.

(B) Typical progressive lesions of fowl pox on thigh of unvaccinated bird (*right*) exposed in parallel with fowl at left. Both fowls were of the same age group and were kept under the same conditions.

(C) Barred Rock chickens two weeks following exposure to 1-percent aqueous suspension of fowl-pox virus via feather-follicle method. Note absence of lesion on thigh of chicken at left, inoculated 34 days preceding exposure to fowl pox with a .5-percent aqueous suspension of skin-lesion pigeon-pox virus, feather-follicle method. Unprotected control at right was kept under same conditions as inoculated bird and was exposed to fowl-pox virus by the same method at the same time.

(D) Rhode Island Reds two weeks following exposure to 1-percent aqueous suspension of fowl-pox virus by feather-follicle method. Note apparent degree of protection afforded bird at left which had been inoculated by feather-follicle method with a .5-percent aqueous suspension of skin-lesion pigeon-pox virus 34 days preceding exposure to fowl pox. Unprotected control at right was kept under same conditions and exposed to fowl pox at same time as vaccinated bird.

(E, F) Photographs taken 20 days after exposure to a 1-percent aqueous suspension of fowl-pox virus. Thirty days before exposure to fowl pox the bird at left was inoculated with 1-percent aqueous suspension skin lesion pigeon-pox virus by feather-follicle method. Note absence of lesions as compared with inoculated bird at right, which was kept under same conditions, was of same age and weight, and was exposed to the fowl-pox virus by same method at same time.



Summary. In this group of experiments, a total of 858 chickens ranging from 3 to 18 weeks of age were vaccinated with pigeon-pox virus by the feather-follicle method. In all groups but one, subsequent exposure to fowl pox was artificial and consisted of liberal application of a 1-percent aqueous suspension of fowl-pox virus to the scarified combs and feather follicles. The pigeon-pox vaccinated fowls were exposed at periods varying from 14 to 180 days following vaccination.

A definite folliculitis at the site of application of the pigeon-pox virus was regarded as a take. These reactions reached their height at about the 6th to the 9th day following vaccination, no scabs were formed, and none of the chickens displayed any detectable systemic reaction which might be attributed to the pigeon virus. The conditions under which these experiments were conducted simulate, to some extent, those which might prevail upon premises potentially harboring fowl-pox infection.

Consequently the possibility that an unobserved mild type of natural fowl-pox infection occurred in some of the experimental fowls must be considered, altho the marked lesions and systemic reactions observed in the controls, as compared with the mild receding lesions and the absence of systemic disturbances in the vaccinated birds, do not lend support to this hypothesis. In several of the experiments the control fowls were left in the same pen with those which had received pigeon-pox virus and in only a negligible number of instances were the controls refractory to artificial infection with fowl-pox virus. The significant results of these tests may be summarized as follows:

1. Chicks ranging from 3 to 18 weeks of age when vaccinated by the feather-follicle method with a 1-percent aqueous suspension of pigeon virus (Illinois strain) displayed a measurable but incomplete degree of protection when artificially exposed to fowl-pox virus at varying periods of time following vaccination (Fig. 5).

2. The resistance induced in young chickens by the pigeon strain of virus appeared to be of a uniform nature during the first two months following vaccination. Beginning about two months after vaccination, there appeared to be a gradual diminution in the degree of resistance in many of the fowls, as judged by the results following artificial exposure to fowl virus.

3. In one experiment a 1/2-percent aqueous suspension of pigeon-pox virus induced a degree of resistance in 8-week-old chicks comparable to that observed following the use of a 1-percent suspension of the same virus.

4. The pigeon virus, stored at ice-box temperature (12 to 15° C.) for 14 months in the form of powdered scabs, induced few takes and no measurable resistance when a 1-percent aqueous suspension was applied by the feather-follicle method.

5. Chickens vaccinated at 16 weeks of age with 1-percent aqueous pigeon-pox virus and naturally exposed to fowl pox 21 days after vaccination showed no evidence of becoming infected over a period of 30 days.

6. There appeared to be no difference in the degree of resistance to fowl pox induced in chickens of different breeds by the use of pigeon virus.

7. No undesirable results were observed in any of the chickens as a result of vaccination with pigeon virus.

8. Some fowls appeared measurably resistant to artificial exposure to fowl pox for as long as six months following vaccination with the pigeon-strain virus applied by the feather-follicle method.

9. As determined by a single experiment, there appeared to be no difference in the results of two methods of "mixing" the pigeon-pox virus with the vehicle or suspending medium so long as a fairly uniform suspension was effected.

Application by stick method. Reports on the use of pigeon virus in chickens show that the feather-follicle method of application has been used almost exclusively. In a few instances, however, the stick method has been employed. Johnson (1930) vaccinated chickens with pigeon virus by the stick method and reported the production of consistent takes. This was interpreted to favor the use of the stick method, but later Johnson (1934) concluded that pigeon virus applied by one stick per fowl gave slight, if any, protection against artificial or natural exposure to fowl virus. Furthermore, he obtained evidence that a considerable increase in the number of vaccination points, both stick and follicle, failed to give desired protection. Broerman and Edgington (1932) stated that pigeon-virus vaccination by one stick and by one feather follicle failed to produce immunity in chickens to fowl virus administered three to four months later. Orr and Emmel (1933), however, used pigeon virus by the stick method for vaccinating 80 pens of chickens in an egg-laying contest, and concluded that the results were generally satisfactory.

As previously reported by Graham and Barger (1935), evidence has been obtained to suggest that the feather-follicle method may have advantages over the stick method as a means of applying pigeon virus to chickens. Table 2 gives the data on this phase of the experiments with the Illinois strain of pigeon pox.

Procedure. In every case a freshly prepared 1-percent aqueous suspension of powdered pigeon-pox virus was used. A long narrow-bladed scalpel with a sharp point was used for piercing the comb or the under-surface of the patagium. In a few instances a 14-gauge hypodermic needle was employed as the piercing instrument. The puncture of the patagium was made with the tip of the scalpel after it had been dipped in the virus suspension. In a few cases the virus was applied to the

comb. After numerous scarifications had been made on both sides of the appendage, the virus was rubbed on with a cotton swab dipped in the virus suspension.

It was found difficult in many instances to determine whether or not a reaction to the virus had occurred. Small scabs were present in a majority of cases, but unless there was a characteristic reaction at the site of puncture, the scabs were attributed to traumatism and were not considered to be true responses to the virus (Fig. 6). Swellings and scab formations regarded as virus reactions were found to vary in the length of time they persisted, but as a rule there was complete subsidence within 15 days after application.

All of the surviving chickens treated with pigeon virus in these experiments were ultimately exposed to fowl pox. Unless otherwise indicated, exposure to fowl pox was severe and consisted of applying a 1-percent aqueous suspension of fowl virus to the scarified combs, or to the feather follicles on the leg, or to both areas.

Results. Four of the five chickens from Lot 1, exposed at 30 days, developed moderate lesions (Table 2). The survivors at 180 days developed lesions as severe as those in the controls. The latter group consisted of birds which served also as controls on another experiment. The controls were exposed with the same suspension of virus as used for the vaccinated fowls. The results indicate that very little, if any, protection to severe pox exposure was afforded by the application of pigeon virus by the stick method as herein employed.

Coccidiosis was responsible for heavy mortality in Lot 2. Of the survivors only 8 birds were used at each exposure interval. Evidence of protection against fowl virus was not observed. The possibility that

TABLE 2.—RESULTS OF PIGEON-VIRUS VACCINATION AGAINST FOWL POX IN CHICKENS: STICK METHOD

Lot	Number of chickens	Breed*	Age	Vaccination reaction	Number exposed	Days from vaccination to exposure	Results
1.....	61	BR	4 wks.	Doubtful	5	30	Partial immunity in 4
1C.....	54	Mixed	3-6 wks.	19	180	No immunity
					54	...	No immunity
2.....	142	BR-R1R	1-2 days	Doubtful	8, 8, 8	26, 60, 90	No immunity
2C.....	8	R1R	1-2 days	8	...	No immunity
3.....	30	R1R-BR	5-9 wks.	20 takes	30	16	No immunity
		WL-C	5-9 wks.	10 negative			
4.....	6	WL-C	5-9 wks.	Doubtful	6	70	No immunity
5.....	50	WL-C	5-9 wks.	Doubtful	50	112	No immunity
6.....	29	WL-C	5-9 wks.	Doubtful	29	133	No immunity
7.....	282	R1R-BR	3-5 wks.	Doubtful	20, 20	30, 60	Doubtful immunity
7C.....	13	BR-R1R	3-9 wks.	20, 20, 15	90, 120, 150	Partial immunity
					13	...	Partial immunity

*BR = Barred Rock; R1R = Rhode Island Red; WL = White Leghorn; C = Cornish.

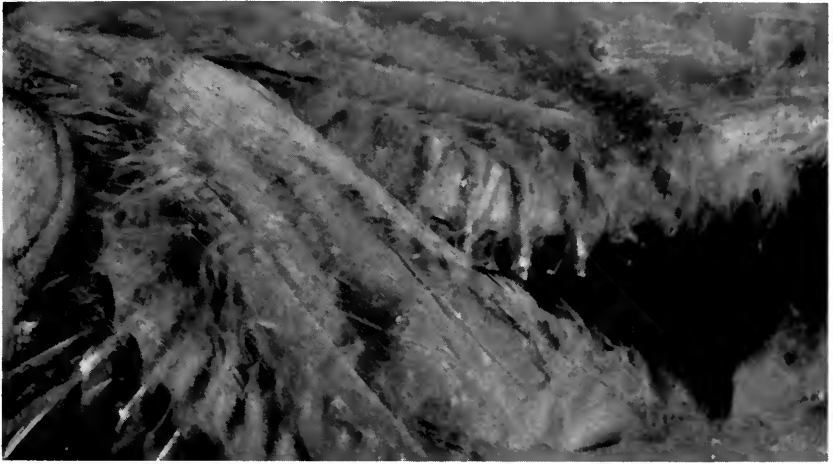


FIG. 6.—TRAUMA, OR NEGATIVE TAKE, ON INNER SURFACE OF RIGHT WING OF TWO CHICKS VACCINATED AT ONE MONTH OF AGE, STICK METHOD

A 1-percent aqueous suspension of skin-lesion pigeon-pox virus was used. Photograph was taken eight days following inoculation.

failure to develop immunity was the result of coccidiosis was not excluded, altho other observations indicate that an attack of coccidiosis concurrent with the use of fowl-pox virus in chicks may not prevent the development of immunity. Furthermore, coccidiosis-free young birds (Lots 3, 4, and 5) treated by the same method also failed to develop significant immunity to artificial exposure.

The chickens in Lots 3 to 6 inclusive were taken at random from a flock of 2,549 birds treated with pigeon virus at 5 to 9 weeks of age. The reactions to the pigeon virus were doubtful in most cases, altho slight swellings were occasionally observed at the point of puncture. The birds removed at intervals up to 112 days did not show appreciable resistance to severe artificial exposure; and of the birds in Lot 6, removed for testing at 133 days, over half contracted fowl pox as a result of exposure to pen infection.

In Lot 7 the results in birds exposed at 30 to 60 days following treatment with pigeon virus are excluded from consideration because of the high mortality from coccidiosis and because of failure of the controls in these groups to develop fowl-pox lesions. In the other three groups of this lot, exposed at 90, 120, and 150 days, there was evidence of partial resistance, since the lesions and reactions were mild as compared with those in the control birds. However, the possibility that the birds in Lot 7 had acquired resistance as the result of unrecognized infection cannot be entirely disregarded.

Summary. Pigeon virus, as applied by the single-stick method in these experiments to more than 3,000 chickens ranging from 1 day to 9 weeks of age failed to induce an appreciable or measurable resistance in 675 fowls later exposed to fowl pox at intervals of 16 to 180 days. In the majority of cases it was difficult to determine satisfactorily whether the local reactions following treatment by the stick method were true takes or merely the result of traumatism. In only one instance was evidence suggested that the virus had possibly induced a measurable degree of resistance to fowl pox. In that group, however, the reactions were so irregular as to render doubtful any assumption that the virus was responsible for the resistance manifested upon later exposure. In one flock of more than 2,500 young fowls the pigeon virus failed to induce any resistance, as judged by the severe lesions and reactions in fowls later artificially exposed to fowl pox at varying intervals of time.

Parallel tests by feather-follicle and stick methods. To check the results in the tests reported in the preceding sections, the stick and feather-follicle methods of applying pigeon-pox virus were employed in parallel for comparative purposes. Chicks of the same breed, age, and source were utilized. The results of these and subsequent experiments conducted in this laboratory would seem to indicate that the method of feather-follicle vaccination employed resulted almost invariably in a greater degree of exposure, with a corresponding greater response, than did the stick method of application. The results of these parallel experiments with pigeon virus are given in Table 3 and illustrated in Figs. 7 and 8.

Vaccination of Lots 1 and 3 by the stick method and Lots 2 and 4 by the feather-follicle method was done with the same freshly prepared 1-percent suspension of pigeon virus and according to the procedure previously described. Exposures to fowl pox consisted of the application of 1-percent aqueous suspensions of fowl virus to the scarified combs and the open feather follicles of the leg.

Results. Upon examination for takes in Lot 1 (stick method), all reactions were recorded as doubtful or questionable. In contrast, all birds except 2 in Lot 2 (feather-follicle method) showed definite takes following application of pigeon virus. Upon exposure to fowl pox at 30 and 60 days following treatment, evidence of immunity was not demonstrated in 20 birds each from Lot 1 and Lot 1C, the control group, while all the birds from Lot 2 manifested a definite partial immunity. At 225 days after vaccination a partial immunity, probably acquired, was demonstrated in the treated birds as well as in the controls. The birds of Lots 3 and 4 yielded virtually the same results upon exposure to fowl pox as did Lots 1 and 2, and the birds in Lots 3C and 4C, like most of those in Lots 1C and 2C, showed no immunity.

TABLE 3.—RESULTS WITH PIGEON VIRUS APPLIED BY THE STICK AND THE FOLLICLE METHODS

Lot	Number of chicks	Breed	Age	Method	Vaccination reaction	Number exposed	Days from vaccination to exposure	Results
1.....	70	BR	<i>weeks</i> 4	Stick	Doubtful	20 20 6	30 60 225	No immunity No immunity Partial immunity
2.....	70	BR	4	Follicle	Folliculitis	20 20 6	30 60 225	Partial immunity Partial immunity Partial immunity
1C, 2C.....	70	BR	4	20 20 17	No immunity No immunity Partial immunity
3.....	100	BR	4-6	Stick	Negative	20	30	No immunity
4.....	100	BR	4-6	Follicle	Folliculitis	20	30	Partial immunity
3C, 4C.....	100	BR	4-6	20	No immunity



FIG. 7.—EFFECT OF VACCINATION WITH A 1-PERCENT AQUEOUS SUSPENSION OF SKIN-LESION PIGEON-POX VIRUS SIMULTANEOUSLY APPLIED BY FEATHER-FOLLICLE AND STICK METHODS

Thirty days after vaccination the two inoculated birds and the control bird were exposed to fowl pox by applying a 1-percent aqueous suspension of fowl-pox virus to the scarified comb. Twenty-one days following exposure to fowl-pox virus this photograph was made. Note protection induced by lesion pigeon-pox virus applied feather-follicle method (1) in contrast with failure of pigeon virus to protect when applied stick method (2). The control bird is at the right (3). (Numbers on photograph do not coincide with lot numbers in Table 3.)

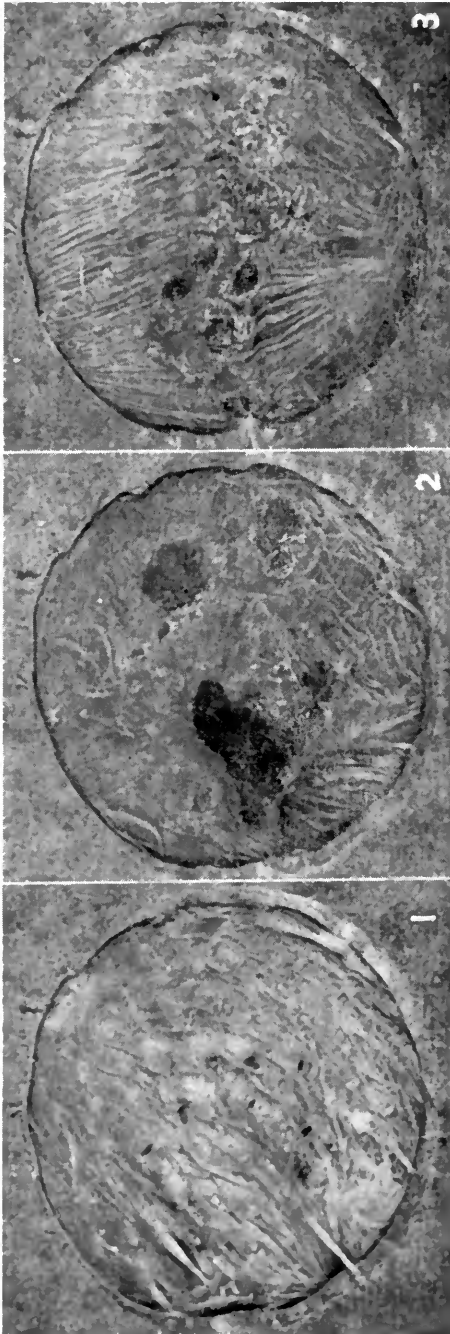


FIG. 8.—PROTECTIVE CHARACTER OF PIGEON-POX SKIN-LESION VIRUS APPLIED BY FEATHER-FOLLICLE METHOD, CONTRASTED WITH THAT APPLIED BY STICK METHOD

A 1-percent aqueous suspension of pigeon-pox skin-lesion virus had been applied via feather-follicles to bird at left and via stick method to bird in center. One month later these birds and the control or unvaccinated bird (*right*) were exposed via feather follicles to a 1-percent aqueous suspension of fowl-pox virus. These photographs were made 18 days following exposure to fowl-pox virus. Note receding lesions in bird at left, in contrast to progressive lesions in the other two.

Conclusions. The temporary partial immunity to fowl pox induced by the feather-follicle method of vaccination with pigeon virus, as contrasted with the doubtful or negative immunity response following the stick method, may be attributed largely to the larger area of inoculation and increased degree and extent of reaction following the feather-follicle application compared with the smaller area of inoculation and mild reaction induced by the stick method. The findings in this experiment, together with those in other experiments conducted in this laboratory, appear to lend support to the opinion expressed by De Blicke (1925), Johnson (1934), Basset (1935), and others, viz., that in general the degree and duration of immunity to fowl pox resulting from pigeon-pox virus vaccination are directly dependent upon the degree or extent of the cutaneous reaction induced by the vaccination, or to both these factors.

Antidiphtherin Vaccination Against Fowl Pox

Source of antidiphtherin. In 1933 two vials of antidiphtherin were obtained from De Blicke for the purpose of studying its value as an immunizing agent against fowl pox in chickens. The substance was a flaky light-brown powder which produced a somewhat milky solution when suspended in water. No directions for its preparation accompanied the product.

De Blicke had stated (1927) that "antidiphtherin contains living matter, which, however, perishes in a few days when out of the refrigerator; the vaccine can, however, be preserved for months at minus 10° C." The viability of the 1933 shipment of vaccine after it was received in the United States was therefore considered questionable, as the temperature of the product en route was doubtless unfavorable. The exact time during which the antidiphtherin was out of refrigeration en route is not known, but probably it was about two weeks.

Immediately upon receipt of the antidiphtherin, a 1-percent aqueous suspension was prepared and swabbed over the plucked breasts of pigeons in an effort to propagate the virus. It was found that the virus was active and could readily be maintained by serial passage thru pigeons. It was subsequently propagated by pigeon passage over a period of two years with no detectable loss of virulence. Some of the results obtained in applications of this virus to pigeons and chickens are shown in Figs. 9, 10, and 11.

Application. When applied to the open feather follicles of pigeons, antidiphtherin induced a reaction indistinguishable at first from that characteristic of pigeon-pox infection. There was hyperemia of the skin over the treated area, accompanied by severe folliculitis and the formation of crusts and scabs. After 8 to 10 days there was a tendency

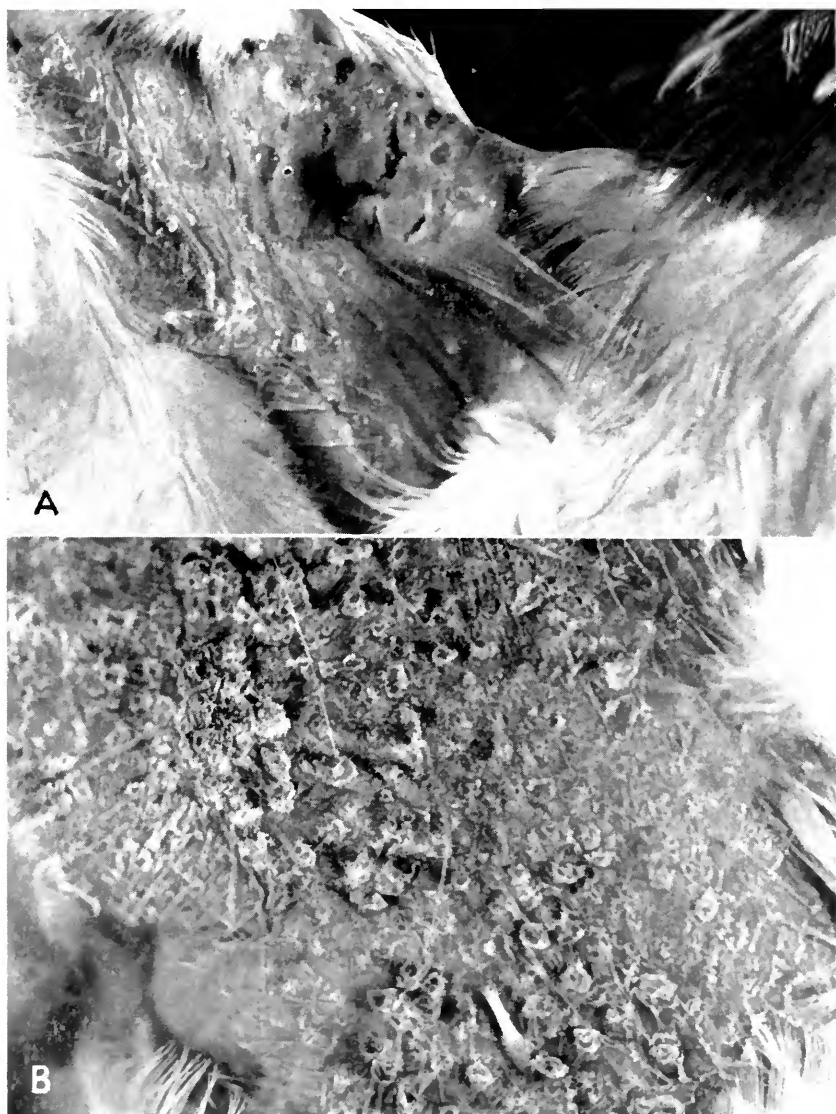


FIG. 9.—INFECTIVE AND ANTIGENIC PROPERTIES OF ANTIDIPHATHERIN APPLIED TO WING AND BREAST OF PIGEON

(A) Note progressive pox lesions on undersurface of pigeon wing 7 days after application of a 1-percent aqueous suspension of antidiphtherin, stick method. (B) Diffuse lesions on pigeon breast 21 days following application of a 1-percent aqueous suspension of antidiphtherin, feather-follicle method.

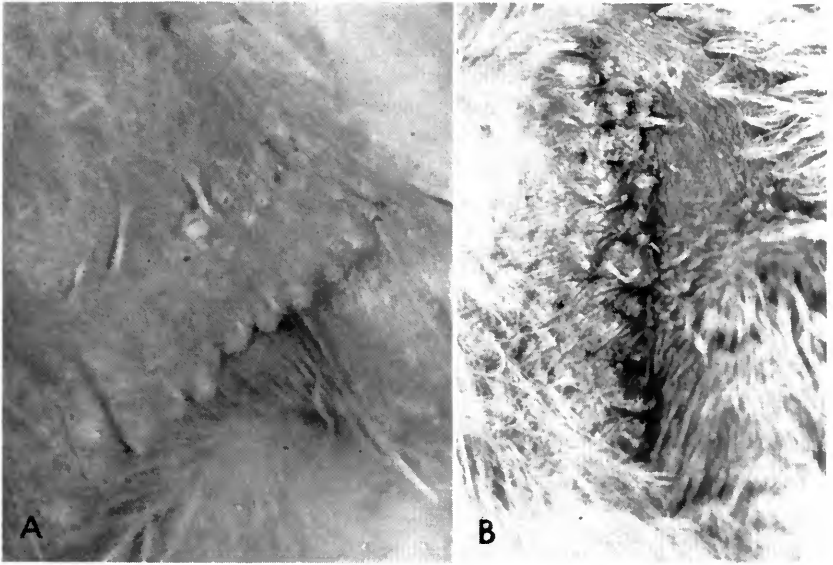


FIG. 10.—INFECTIVE PROPERTIES OF ORIGINAL ANTIDIPHATHERIN APPLIED TO THIGH

(A) Local reaction on right thigh of chicken 9 days following application of a 1-percent aqueous suspension of antidiphtherin via feather-follicle method. (B) Local reaction on thigh of 5-week old White Rock chicken 10 days following application of a 1-percent aqueous suspension of antidiphtherin via feather-follicle method.

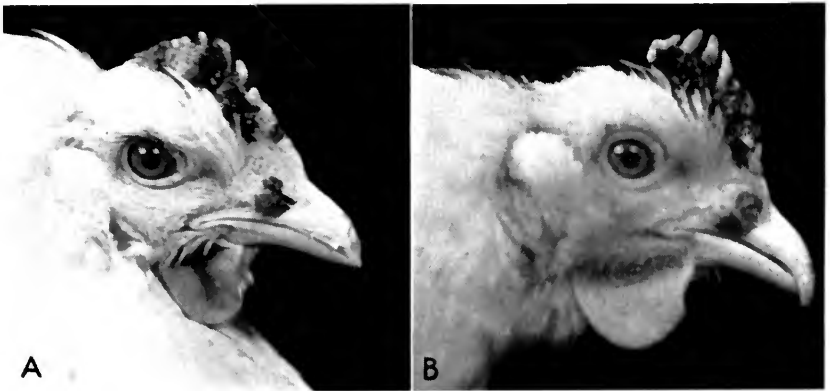


FIG. 11.—FAILURE OF ANTIDIPHATHERIN TO PROTECT AGAINST ARTIFICIAL EXPOSURE TO FOWL-POX VIRUS EIGHT MONTHS AFTER VACCINATION

Chicken at left (A) was vaccinated with a 1-percent aqueous suspension of antidiphtherin via feather-follicle method. Eight months later this bird and the control (B) were exposed to a 1-percent aqueous suspension of fowl-pox virus by scarification of the combs. Photograph taken two weeks after exposure.

TABLE 4.—RESULTS OF EXPERIMENTS ON THE IMMUNIZING PROPERTIES OF ANTIDIPHOTHERIN FOR FOWL POX

Lot	Number of birds	Breed	Age <i>weeks</i>	Vaccination		Vaccination reaction	Number exposed	Days from vaccination to exposure	Results of artificial exposure to fowl pox
				Method	Site				
1.....	2 chickens 2 chickens 2 chickens 6 pigeons	WL WL WL Unknown	6 6 6 Unknown	Stick Stick Follicle Follicle	Comb Wing Leg Breast	Negative Doubtful Positive Severe	2 2 2 0	26 26 26 ...	One immune, one not immune No immunity, severe pox Partial immunity ^b Five died within 21 days
2.....	12 chickens 12 chickens 3 chickens	BR BR BR	4 4 4	Stick (PP) ^a	Wing Wing	Doubtful Doubtful	12 12 3	27 27 ...	No immunity ^c No immunity ^c No immunity ^c
2C.....							20 20, 10 9	30 60, 77 126	Partial immunity ^b Partial immunity ^b Partial immunity ^b
3.....	110 chickens	BR	4	Follicle	Leg	Pos. in 106	15 74 ^f	231 ...	Doubtful immunity ^e No immunity ^e
3C.....	74 chickens	BR	4			No immunity ^e

^aIllinois pigeon virus was used in this test. ^bMild lesions, no systemic reactions. ^cSevere pox lesions and systemic reactions. ^dModerate lesions, slight systemic reactions. ^eSevere lesions, moderate systemic reactions. ^fCorresponding number of controls were exposed with each of the 5 groups of vaccinated stock in Lot 3.

for the scabs to coalesce, the entire area becoming covered with a dry brownish scab formation. At this point there appeared to be a difference in the gross appearance of antidiphtherin lesions compared with those produced by the Illinois strain of pigeon-pox virus in that the former appeared to be drier and more flaky or scaly.

Pigeons heavily inoculated for the propagation of antidiphtherin manifested a severe systemic reaction characterized by droopiness, ruffled feathers, impaired appetite, and loss of flesh. Death usually occurred within 14 to 18 days, but whenever possible the pigeons were destroyed when moribund. The scab skin lesions of the breast were removed and desiccated over calcium chlorid and sulfuric acid for four days at room temperature. They were then cut into small pieces, ground fine in a sterile mortar, sifted thru gauze, and the resulting powder stored in tightly stoppered brown-glass bottles in the refrigerator (8 to 12° C.) as stock vaccine. A freshly prepared 1-percent aqueous suspension was used as the vaccine in the experiments here reported (see Table 4).

Results. It was planned originally to test the immunizing value of antidiphtherin against fowl pox in the chickens and pigeons in Lot 1 (Table 4). However, five of the six pigeons died within three weeks, presumably as the result of severe cutaneous lesions developing from vaccination inoculation of one half of the breast. Obviously, less extensive vaccination should have been employed. The antidiphtherin was a 1-percent suspension of the original lot received from De Blicck.

Only the two chickens treated by the follicle method developed a take in the form of a definite folliculitis. Likewise, these were the only two birds of the lot of six which gave definite evidence of partial immunity as manifested by mild transient lesions without systemic reactions.

The 24 chickens in Lot 2 were divided equally into two groups to compare the immunizing effect, if any, of antidiphtherin (produced from pigeons by inoculation of original material received from De Blicck) and the Illinois strain of pigeon-pox virus. In neither lot was there any definite evidence of a vaccination reaction or of acquired immunity to fowl pox after exposure 27 days following vaccination with these products.

Virtually all of the chickens in Lot 3 showed a definite take as a result of vaccination with 1-percent aqueous suspension of antidiphtherin prepared in this laboratory. Evidence of well-developed partial immunity was apparent at 30, 60, 77, and 126 days following vaccination. At 126 days some immunity was evident but of a diminishing degree. The results of exposure at 231 days suggest that immunity to fowl pox previously present had diminished to a low or insignificant level. In view of later observations in this laboratory, as well as of the statements of Doyle (1933), it seems highly probable that marked and

undesirable attenuation of the potency of the antidiphtherin was brought about by desiccation at room temperature and atmosphere. Consequently exposure of a large area of skin to vaccination would seem to offer greater opportunity for a satisfactory take and immunity.

The results of treating chickens with antidiphtherin, both the original and that subsequently produced on pigeons, were somewhat comparable to those observed when pigeon-pox virus, Illinois strain, was used as a vaccine. Furthermore, when applied by the feather-follicle method, the local reactions induced by 1-percent aqueous antidiphtherin were indistinguishable from those produced by the same concentration of pigeon-pox virus. As with pigeon-pox virus (Illinois strain) it was impossible to determine accurately whether the mild reaction which followed the stick method of application of antidiphtherin was a true response to the virus or merely the result of trauma in puncturing the skin.

No systemic disturbance was induced in chickens by a 1-percent aqueous suspension of antidiphtherin, whether applied by the feather-follicle or stick method. There appeared to be a definite degree of resistance induced in chickens by feather-follicle vaccination of the fowls with 1-percent aqueous suspension of antidiphtherin; but when applied by the stick method, the same vaccine did not produce a significant take or measurable resistance to fowl pox. Antidiphtherin would therefore seem to resemble closely the Illinois strain of pigeon virus in its potency and antigenic properties for chickens.

Summary. 1. Antidiphtherin supplied to the Illinois Agricultural Station by Dr. De Blicke of Amsterdam, Holland, proved virulent for pigeons and chickens on arrival at the Laboratory of Animal Pathology and Hygiene, University of Illinois.

2. Antidiphtherin was easily propagated upon the skin of the plucked breasts of pigeons.

3. A 1-percent aqueous suspension of antidiphtherin induced in chickens a folliculitis grossly indistinguishable from that which resulted when the same concentration of Illinois strain of pigeon-virus-infected skin was applied to the feather follicles.

4. A 1-percent aqueous suspension of antidiphtherin applied to chickens by the stick method did not induce a definite local reaction, nor was there any resistance displayed by the fowls vaccinated in this manner upon subsequent artificial exposure to fowl pox.

5. Chickens vaccinated with a 1-percent aqueous suspension of antidiphtherin by the feather-follicle method showed a measurable degree of resistance when artificially exposed to fowl pox at 30, 60, 77, and 126 days following vaccination. When fowls vaccinated with antidiphtherin by the feather-follicle method were exposed to fowl pox at 231 days following vaccination, severe lesions and moderate systemic reactions were produced.

Conclusion. As determined in this experiment, antidiphtherin resembles an Illinois strain of pigeon virus in the gross appearance of the lesions produced in pigeons and chickens, as well as in the degree and duration of induced immunity to fowl pox in these species.

Vaccine Virus in Vaccination Against Fowl Pox: Feather-Follicle Method

The prevailing opinion, as expressed by various investigators, appears to be that the virus of contagious epithelioma and the vaccine virus are not identical and that distinct immunological differences exist between them. The reports of numerous studies and observations which are reviewed in Part I indicate the trend of investigational effort and the deductions of the various workers, particularly in so far as the effect of vaccine virus upon chickens is concerned.

Experiments were therefore carried out to determine whether, by the method employed, vaccine virus would produce a demonstrable degree of resistance to fowl-pox in chickens.

Procedure. The vaccine virus was from a lot being used by the University of Illinois Health Station for the immunization of human beings and, judged by the results, appeared to possess normal virulency. Vaccination of chickens was carried out by applying the undiluted vaccine virus to open feather follicles on the legs of fowl-pox-susceptible chickens. Subsequent exposure to fowl pox consisted of swabbing a 1-percent aqueous suspension of fowl-pox virus over the scarified combs and open feather follicles on the legs.

Results. The results of vaccinating 208 chickens with vaccine virus and subsequently exposing them to fowl pox are shown in Table 5. Vaccination of chickens in Lot 1 was restricted to exposure of only one open feather follicle because of the limited amount of vaccine virus available. Particular care was exercised to insure definite introduction of the virus suspension into the follicle in each case. The fact that not a single vaccination reaction occurred among these 50 17-day-old chicks could be attributed to the probability that the amount of virus was too small or that the virulence had been diminished. Subsequent exposure of the chickens to fowl-pox infection (heavy inoculation of the comb) at 15 to 42 days failed to reveal any evidence of acquired immunity, since the extent and severity of the lesions and the systemic reactions were apparently the same in the vaccinated and the control groups.

In order to check the results obtained in Lot 1, vaccine of another lot was applied to another group of 98 birds 8 weeks of age (Lot 2, Table 5), in the same manner as to Lot 1; that is, to a single feather follicle. Fifty percent of the surviving chicks showed definite folliculitis at the vaccination site. Since the technic of applying the vaccine

TABLE 5.—RESULTS OF VACCINATING CHICKENS AGAINST FOWL POX WITH VACCINE VIRUS APPLIED BY THE FEATHER-FOLLICLE METHOD

Lot	Number of birds	Breed	Age	Vaccination reaction	Number exposed	Days from vaccination to exposure	Results of artificial exposure to fowl pox
1.....	50	BR	17 days	Negative	10	15	No immunity ^a
1C....	36	BR	17 days	26	42	No immunity ^b
					10, 26 ^c	...	No immunity ^a
2.....	98	R1R	8 wks.	Definitely positive in 50%	88	30	No immunity ^a
2C....	93	R1R	8 wks.	93	...	No immunity ^a
3.....	60	BR	4 wks.	Definitely positive in all but 5	20	30	No immunity ^a
3C....	20	BR	4 wks.	20	...	No immunity ^a

^aSevere pox lesions and systemic reactions in all. ^bSeveral chicks died during third week after exposure, presumably as a result of inoculation with fowl pox. ^cCorresponding number of controls were added to each group of vaccinated chickens when exposed.

virus was identical to that used in Lot 1, the takes observed might be most logically ascribed to a more virulent virus than that used in Lot 1. No systemic disturbance was observed in any of the vaccinated fowls. All the birds exposed to fowl pox 30 days following vaccination developed severe pox, there being no discernible difference between the lesions of those fowls that had developed takes and those which had not. The lesions in the vaccinated fowls were as extensive as those in the controls.

A sufficient amount of vaccine virus was available for swabbing several open feather follicles on the leg of each of 60 more birds 4 weeks old (Lot 3, Table 5). The vaccine was freely applied. All but 5 birds showed positive reactions, or takes, consisting of definite folliculitis of the treated area. None of the birds showed a perceptible systemic disturbance. Upon artificial exposure of 20 vaccinated and 20 unvaccinated birds to fowl pox 30 days later, the vaccinated birds were as severely affected as were the controls, indicating that significant resistance had not been induced by heavy vaccination with vaccine virus. Since severe pox lesions developed in the 20 vaccinated birds, it was not deemed necessary to expose the balance of the birds in this lot.

Conclusion. The fact that no appreciable resistance was induced by the vaccine virus in any of the three lots of chickens in this experiment would seem to corroborate the contention that no close immunological relation exists between vaccine virus and fowl-pox virus.

Chemically Treated Pigeon and Fowl Viruses in Vaccination Against Fowl Pox

The various reports dealing with efforts to prepare a satisfactory "true" vaccine against fowl pox emphasize the fact that suitable uni-

TABLE 6.—EFFECT OF CERTAIN AGENTS ON ACTIVITY OF STORED SUSPENSIONS OF PIGEON-POX VIRUS
(Rhode Island Red chicks vaccinated at 6 weeks of age and exposed 28 days later)

Lot	Agent and amount added	Storage temperature for 31 days	Number of chicks	Vaccination reaction	Results on exposure
1.....	10% glycerin.....	Room Ice-box	5 5	Negative Negative	No immunity, severe lesions and systemic reactions in all six lots
2.....	.5% phenol.....	Room Ice-box	5 5	Negative Negative	
3.....	.5% tricresol.....	Room Ice-box	5 5	Negative Negative	
4.....	.5% formalin.....	Room Ice-box	5 5	Negative Negative	
Control.....	Nothing added....	Room Ice-box	5 5	Negative Negative	
Control.....	Not vaccinated....	3	

form attenuation by chemical means is indeed difficult, if not impossible, to attain. Since it is apparent that chemical action brings about a progressive destruction of the total quantity of the virus and not a modification of the virulency of the virus elements, units, or bodies present, such attenuation is comparable to that accomplished by simple dilution. In general, simple physical dilution with a vehicle not actively virucidal would, however, seem to possess certain advantages over the employment of agents quite active in this respect. The potential dangers incident to the use of an unmodified virus in high concentration are well recognized. Nevertheless, the possibility that chemical treatment of a given strain of virus may yield a satisfactory vaccine has perhaps justified continued studies in this direction with fowl pox.

Pigeon-pox virus suspensions. To each of four lots of freshly prepared 1-percent aqueous suspensions of pigeon-pox skin-lesion material, the following agents were added in the concentrations indicated: 10 percent glycerin, .5 percent phenol, .5 percent tricresol, and .5 percent formalin. A fifth lot of the untreated suspensions served as a control. Each of the five suspensions was divided into two parts, one being stored at 10 to 15° C. and the other at room temperature, 20 to 27° C. After being held for 31 days, the virus-treated mixtures were used to inoculate chickens.

The results obtained in inoculation of chickens by scarifying comb and wattles and feather follicles with chemically treated pigeon-pox viruses stored at ice-box and room temperatures, are summarized in Table 6. The chickens in Lots 1 to 4 inclusive were employed to determine the effect of the addition of certain chemicals to an aqueous suspension of pigeon-pox virus upon the viability of the virus, as judged

TABLE 7.—EXPERIMENTS TO DETERMINE THE IMMUNIZING VALUE OF SUSPENSIONS OF FOWL-POX VIRUS TREATED WITH FORMALIN

Lot	Number of chicks	Age	Breed	Vaccine employed	Method	Vaccination reaction	Number exposed	Days from vaccination to exposure	Results
1.....	40	<i>weeks</i> 5	BR	1% suspension in 20% glycerin + .2% formalin	Scarification of comb and feather follicle	Negative	20 12	30 60	No immunity No immunity
1C.....	40	5 5	BR BR	20 20	30 60	No immunity No immunity
2.....	40	5-10	BR	10% suspension in 20% glycerin + .2% formalin	Follicle	Negative	9 ^a	20	No immunity
2C.....	8	5-10	BR	8	...	No immunity
3.....	32	4	BR	1% suspension + .2% formalin	Follicle	Negative	27	30	No immunity
4.....	32	4	BR	1% suspension + .15% formalin	Follicle	Negative	28	30	No immunity
5.....	32	4	BR	1% suspension + .1% formalin	Follicle	Negative	30	30	No immunity
6.....	32	4	BR	1% suspension + .05% formalin	Follicle	Negative	30	30	No immunity
7.....	32	4	BR	1% suspension + .025% formalin	Follicle	Negative	30	30	No immunity
8.....	32	4	BR	1% suspension	Follicle	Positive	0 ^b

^aAn outbreak of laryngotracheitis which developed immediately after vaccination decimated this lot, leaving only 9 for exposure at the 20th day. ^bThe purpose of Lot 8 was to test the virulence of the untreated virus suspension. The reaction to vaccination was severe, and the birds were not exposed at all.

by takes following its application and the immunity induced in treated fowls.

None of the birds treated with the four chemically treated virus suspensions showed definite takes, and upon exposure to fowl pox 28 days after treatment all developed fowl pox as severe as that in the control birds.

It is apparent that neither the treated nor untreated suspensions of pigeon-pox scabs had retained the capacity to incite local takes and subsequent partial immunity to fowl pox. Brunett (1933) demonstrated that 20 percent glycerin was satisfactory as a preserving agent for pigeon virus, altho 50 percent and undiluted glycerin were quite destructive. It is therefore apparent that the pigeon virus employed in the present experiments was of low concentration or of a very low order of viability. However, Pyle (1926, 1929) suggested that glycerin in concentration of 40 percent may inactivate fowl-pox virus employed for cutaneous vaccination in 25 days or less, while Brandly and Bushnell (1932) found that glycerin in concentrations sufficient to destroy bacteria present in scab lesion suspensions either markedly attenuated or completely destroyed the pox virus.

Fowl-pox virus suspensions. The results of vaccination experiments employing suspensions of fowl-pox virus treated with formalin are summarized in Table 7.

One- and 10-percent suspensions of freshly collected and dried fowl-pox virus scabs were made in 20-percent glycerin, to which .2 percent of formalin had been added. A 1-percent aqueous solution of the pox scabs was divided into six parts, and formalin was added to the respective parts to make a final concentration as follows: .2, .15, .1, .05, .025 percent; the other portion was left as an untreated control.

After the addition of formalin each lot of virus was stored in the ice box (10° to 15° C.) until used for vaccination. The virus for Lot 1 was stored for 72 hours; for Lot 2, 96 hours; and for Lots 3 to 8 inclusive, 48 hours. Exposure of all lots except Lot 8 was made by applying liberal amounts of a 1-percent aqueous suspension of fowl-pox virus to the scarified comb or skin. Lot 8 was not exposed because the vaccination reaction was severe and the purpose of this lot was to test the virulence of the virus suspension before formalin was added.

Summary. The results of these experiments again confirm the observation recorded by various other investigators, that formalin in low concentrations rapidly inactivates fowl-pox virus. No vaccination reactions were induced by any of the chemically treated fowl-pox and pigeon-pox vaccines employed in this experiment. The treated fowls were subsequently found to be highly susceptible to fowl pox. The virulence of the fowl-pox virus used was established prior to the time of formalin treatment by the production of severe pox in susceptible chickens. The high virucidal activity of formalin for fowl-pox virus

in the form of lesion material and the relatively rapid inactivation of the pigeon-virus suspensions employed, even when kept at low temperature, are emphasized by the results of these tests, which may be briefly stated as follows:

1. One-percent aqueous suspensions of pigeon-pox virus stored at ice-box and room temperatures for 31 days induced neither demonstrable takes nor resistance when used to vaccinate 6-week-old chicks.

2. The addition of 10-percent glycerin did not preserve the virulence of the virus at ice-box or room temperature.

3. Formalized fowl-pox virus prepared by suspending 1 percent of ground desiccated scabs in 20-percent glycerin, adding .2-percent formalin, and holding at ice-box temperature for 72 hours, failed to induce any reaction or resistance to pox in 5-week-old chicks.

4. Formalized fowl-pox vaccine prepared by suspending 10 percent of ground desiccated scabs in 20-percent glycerin, adding .2-percent formalin, and holding at ice-box temperature for 96 hours, did not induce any reactions or resistance to pox in chicks 5 to 10 weeks of age.

5. Formalized fowl-pox vaccine prepared by adding .2-, .15-, .10-, .05-, and .025-percent formalin, respectively, to different lots of 1-percent aqueous suspension of fowl-pox virus and holding at ice-box temperature for 48 hours, failed to induce any reaction or resistance to pox in 4-week-old chicks.

Fowl-Pox Immunization of Day-Old Chicks With Fowl-Pox Virus

Chickens of all ages are susceptible to fowl pox. Well-developed lesions of the naturally contracted disease have been observed in chicks as young as 12 days. In this laboratory the observation of Woodruff and Goodpasture (1931) that chicken embryos inoculated with fowl-pox virus during incubation may show marked pox lesions before, at, or soon after hatching, have been confirmed. Furthermore, mature or even aged fowls, if previously unexposed, may prove susceptible to the infection. In this country reports have been made only occasionally of fowl-pox infection causing severe losses in young chickens in batteries or in other environments. However, Bice (1933) in Hawaii found that pox may become a hazard within a few weeks after hatching because of the high prevalence of mosquitoes which act as vectors. Partly as a result of this, the advisability of vaccinating baby chicks has been suggested, and investigations of this phase are reported by Johnson (1927, 1934), Danks (1931), Sherwood (1932), Dunn and Sherwood (1933), Lourens (1933), Lubbehusen *et al.* (1930, 1937); Devolt *et al.* (1936); and others.

Source and preparation of virus. The fowl-pox virus employed in these tests was prepared by inoculating the scarified combs of young susceptible Leghorn cockerels with a suspension of powdered skin-

pox lesion. The strain employed was one originating in a field outbreak of fowl pox in chickens in Illinois. The scabs from well-developed lesions were harvested 10 to 15 days after inoculation, and these scabs were subsequently dried and prepared for use as vaccine in a manner identical with that employed for the pigeon virus.

Procedure and results. In a preliminary report dealing with the studies here recorded, Graham and Barger (1936A) concluded that vaccination of vigorous day-old chicks may contribute to subsequent severe systemic reactions and high mortality. In support of this statement were the results obtained with two different groups of 1-day-old Barred Rock chicks from pullorum-negative stock vaccinated by the stick method (2 sticks into wing web) with 1-percent aqueous suspension of fowl-pox virus. Records of the losses were kept and the general condition of the chicks noted from time to time. Of 216 chicks in the first lot, 3 died during the first week and 2 during the second week following vaccination. Thereafter the mortality increased markedly with a total loss of 79 chicks during the period of one month following vaccination. Many other chicks showed secondary pox, and a majority of those which eventually recovered were stunted or unthrifty.

In a second lot of 237 Barred Rock chicks from pullorum-tested flocks which were treated in a similar manner when one day old, 19 chicks died during the first week and the total mortality during the month subsequent to vaccination was 107 chicks. Generalized pox and stunted unthrifty chicks were common, as in Lot 1.

Summary and conclusions. 1. The vaccination of 453 day-old chicks from pullorum-negative stock by the stick method was followed within one month by a mortality of 186 chicks (41 percent). In many cases generalized pox developed and the survivors, as a group, were stunted and unthrifty.

2. Severe inoculation fowl pox and general unthriftiness were common.

3. The danger of introducing fowl-pox infection upon contaminated premises by this procedure is quite apparent.

4. The disastrous results of the experimental vaccination of baby chicks with fowl-pox virus in this instance would emphasize the potential danger of the practice.

Pigeon-Pox Immunization With Fowl-Pox Virus

Outbreaks of pigeon pox are apparently of infrequent occurrence in the United States, as judged by the small number of reports to be found in the literature. However, two rather serious outbreaks came to the attention of the Illinois Agricultural Experiment Station in 1930 (Graham and Barger, 1932), while Stafseth (1931) reported

an outbreak in Michigan. Pyle (1932) states that pox is quite prevalent among pigeons and not infrequently results in severe losses. A review of available literature (Part I) did not yield many reports of attempts to induce immunity against pigeon pox in pigeons. The studies reported herewith present only the results of preliminary observations made at this Station.

It has been shown that the Illinois strain of pigeon-pox virus rather constantly induces a mild local reaction when applied to open feather follicles of susceptible chickens, and further that fowls showing such a reaction subsequently manifest a measurable but incomplete resistance to artificial fowl-pox infection. Altho in the experience of the authors a solid immunity is produced in pigeons by vaccinating them with pigeon-pox virus, the reactions, both local and systemic, are often so severe as to render the procedure dangerous and impractical. Attention was therefore directed to a study of the possible value of a strain of fowl-pox virus (apparently monopathogenic) as an immunizing agent against pigeon pox in pigeons.

Procedure. The pigeons used in these experiments were purchased on the open market; their history with reference to previous pox infection was not known. A 1-percent aqueous suspension of fowl-pox virus known to be highly virulent for chickens was employed. Inoculation consisted of the brisk application of the suspension to approximately 10 to 15 open feather follicles on the leg, with two exceptions: one in which it was applied to the plucked breasts, and another in which the stick method was employed. Subsequent test exposure to pigeon pox was artificial in all cases and was effected by swabbing a 1-percent aqueous suspension of pigeon-pox virus into open feather follicles on the breast or on the leg opposite that used for vaccination. The virulence of both the fowl- and pigeon-pox viruses employed was established for susceptible birds.

Results. Altho preliminary exposure tests indicated a very high degree and percentage of susceptibility in pigeons purchased on the open market, a representative number of the birds from each of the ten lots was not tested for pigeon-pox susceptibility prior to the vaccination procedure. Since the possibility of previous unknown pigeon-pox infection with a consequent acquired immunity could not be excluded, the comparative effect of pigeon and fowl pox in producing immunity to pigeon-pox infection must be determined from some of the other experiments here described.

A uniform definite resistance to pigeon pox was demonstrated after 46 days in all pigeons of Lot 2 vaccinated with the pigeon virus. Altho negative or moderate resistance was apparent in half the birds exposed from Lot 2A, treated with fowl virus, the other half developed severe pox when exposed to fowl pox 46 days after vacci-

nation had been attempted. As in Lots 1 and 1A, the possibility of previous exposure and infection with pigeon pox was not excluded in the case of these birds. The very mild or negative response of the pigeons in Lots 1A and 2A to the fowl virus, as compared with the severe or moderate reaction to pigeon-pox-virus vaccination in Lots 1 and 2, suggested that the immunity response to the fowl-pox vaccination would likewise be very mild or negative, as it proved to be.

The results in Lots 3 and 3A are also inconclusive altho, unlike the previous experiments, a well-defined post-vaccination reaction was recorded in Lot 3A. The results of exposing the pigeons in Lot 4 to pigeon virus 30 days after they had been treated with fowl-strain virus may be interpreted to indicate that immunity of a significant degree was not produced.

The results of Experiment 5 (3 lots) would seem to verify the tentative assumption from previous observations that treatment with certain strains of virulent fowl pox fails to produce in the pigeon a satisfactory take or immunity to pigeon virus. All 40 of the treated birds in Lots 5 and 5A developed lesions and systemic reactions as severe as those in the controls of Lot 5C. The irregular results obtained in susceptibility or resistance tests in Lot 6, as well as in previous experiments, seem to warrant the conclusion that some of the pigeons used were refractory to pigeon virus prior to vaccination.

Because of the possibility of pigeon-pox infection prior to purchase or an undetected mild infection during the relatively long period elapsing from the vaccination procedure to exposure, the results observed in Lots 7 and 7C are inconclusive, while Lots 8, 8C, 9, 9C, 10, and 10C yielded evidence of the failure of fowl-pox virus applied by the feather-follicle method to protect pigeons subsequently exposed to pigeon virus.

As summarized in Table 8, evidence was not obtained to indicate that the application of the fowl strain of virus, feather-follicle method, augmented the natural or normal resistance of pigeons to pigeon pox. Of interest in this experiment was the irregular susceptibility of the control birds, this phenomenon in previous experiments having been observed almost exclusively in the treated pigeons.

The results in Lots 9 and 9C substantiate the evidence already accumulated that 1-percent aqueous suspensions of the strain of fowl-pox virus used in this experiment failed to induce any demonstrable resistance in pigeons subsequently exposed to pigeon pox at 30, 60, and 90 days following vaccination.

The results in Lots 10 and 10C confirmed the results in Lots 9 and 9C.

Summary. A total of 355 pigeons, purchased on the open market, were used to determine the comparative ability of one strain each of virulent pigeon-pox virus and fowl-pox virus to produce immunity

TABLE 8.—RESULTS OF IMMUNIZATION EXPERIMENTS AGAINST PIGEON POX IN PIGEONS

Lot	Number of pigeons	Virus	Vaccination reaction	Vaccination method	Number exposed	Days from vaccination to exposure	Results
1.....	17	Pigeon	Moderate in 6, severe in 11	Follicle (leg)	4, 13	42, 49	Very mild lesions, no systemic reactions
1A.....	17	Fowl	Very mild in 15, negative in 2	Follicle (leg)	14*	49	Very mild lesions, no systemic reactions in 13; one was severely affected and died on the 23d day
2.....	8	Pigeon	Severe in all	Follicle (leg)	8	46	Mild lesions, no systemic reactions
2A.....	8	Fowl	Very mild (1 survivor)	Follicle (leg)	6 *	46	Severe lesions in 3, moderate in 1, negative in 2
3.....	6	Pigeon	Severe in 5, mild in 1	Follicle (leg)	6	54	Very slight lesions, no systemic reactions
3A.....	6	Fowl	Well-defined in 5, moderate in 1	Follicle (leg)	6	54	Very slight lesions, no systemic reactions in 5; severe lesions and systemic reaction in 1
4.....	6	Fowl	Mild in 5, negative in 1 ^b	Follicle (breast)	5	30	Two died during 2d week, severe local and systemic reactions in 3 survivors
5.....	20	Fowl	Severe (largely traumatic)	Stick (wing)	17	30	Severe lesions and systemic reactions in all
5A.....	20	Fowl	Mild in all	Follicle (leg)	17	30	Severe lesions and systemic reactions in all
5C.....	4	4	...	Severe lesions and systemic reactions in all
6.....	40	Fowl	Mild in 34, negative in 6	Follicle (leg)	19	30	Severe pox in 6, mild in 7, negative in 6
					15	60	Severe pox in 7, mild in 5, negative in 3
6C.....	10	5 added to 1st group	...	Severe pox in all
					5 added to 2d group	...	Severe pox in 4 survivors
7.....	20	Fowl	Mild to moderate	Follicle (leg)	12	180	No lesions or reactions in any
7C.....	2	2	...	Severe lesions and reactions in both
8.....	75	Fowl	Mild in 59, negative in 8, 8 died	Follicle (leg)	10, 10, 10	30, 60, 90	Severe lesions and reactions in all
8C.....	6	2 added to each group in Lot 8	...	Severe pox in 3, negative in 3
9.....	39	Fowl	Mild in all	Follicle (leg)	5	30	Severe pox in 3 survivors
					10, 11	60, 90	Severe pox in all
9C.....	17	5 added to 1st group	...	Severe pox in 3, mild in 1, 1 died
					5 added to 2d group	...	Severe pox lesions and reactions in all
					7 added to 3d group	...	Severe pox lesions and reactions in all
10.....	19	Fowl	Mild in 9, negative in 10	Follicle (leg)	5, 10, 3	30, 60, 90	Severe pox in all
10C.....	15	5 added to each group	...	Severe pox in all

*Died from undetermined causes during the interval between vaccination and exposure. ^bThis bird died before time for exposure.

to subsequent exposure to the Illinois strain of pigeon-pox virus. Two hundred fifty of these pigeons were treated with fowl-pox virus by the feather-follicle method and 20 by the stick method. Thirty-one were vaccinated with pigeon-pox virus by the feather-follicle method and 54 unvaccinated pigeons were used as controls.

At the outset, the results obtained in Lots 1, 1A, 2, 2A, 3, and 3A suggested that application of a strain of fowl-pox virus, feather-follicle method, had induced a measurable degree of immunity to the Illinois strain of pigeon-pox virus, but additional tests, adequately controlled and including trials with the stick method, indicated that no appreciable resistance had been produced with the fowl-pox virus. That some of the pigeons purchased on the open market possessed an acquired immunity prior to vaccination seemed apparent, but, even if such were not the case, the results of all the experiments suggest that the fowl-pox virus used was not effective in conferring a measurable immunity against the Illinois strain of pigeon virus. On the other hand, the response to vaccination as well as to subsequent exposure indicated that the strain of pigeon virus employed stimulated a definite and uniform immunity to the same strain of virus.

Conclusion. The application of a strain of fowl-pox virus to pigeons, either by feather-follicle or stick method, failed to induce a measurable degree of resistance to subsequent exposure to an Illinois strain of pigeon pox. Pigeon-pox virus applied to a number of open follicles stimulated a definite immunity to pigeon virus, but was frequently associated with severe systemic reactions, sometimes terminating fatally.

Fowl-Pox and Pigeon-Pox Viruses in Vaccination of Pullorum-Exposed Chicks

During the course of the immunization experiments against fowl pox, a number of 3-week-old chickens which had survived exposure to pullorum-disease infection when one day old became available, and it was thought desirable to attempt determinations of the comparative effects of vaccination with fowl-pox and pigeon-pox viruses.

The results of various reported observations and studies seem to establish the fact that the vaccination with unattenuated fowl-pox virus may create a hazard to normal health, development, and function. In birds of a given age this danger appears to be directly proportional to the severity of the response to vaccination and the influence of various devitalizing factors acting concurrently.

Prior to the beginning of this experiment in 1933, several workers, including Van Heelsbergen (1925), Johnson (1927), Beach (1929), Sawyer and Hamilton (1929), Stafseth (1931 and 1931A), Glover (1931), Danks (1931), Lubbehusen and Ehlers (1932), and others,

TABLE 9.—EFFECT OF PIGEON- AND FOWL-VIRUS VACCINATION ON CHICKS SURVIVING EXPOSURE TO PULLORUM DISEASE

Lot	Number of birds	Virus used in vaccination	Losses following vaccination					Total mortality	
			1st wk.	2d wk.	3d wk.	4th wk.	5th wk.	Number	Percent
1.....	110	Pigeon-pox	5	5	0	0	1	11	10.0
2.....	110	Fowl-pox	5	8	41	22	6	83	75.4
Control....	110	8	1	1	0	0	10	9.09

had pointed out that undesirable post-vaccination reactions may follow the use of living fowl-pox virus in chickens of various ages. These reactions were recognized often to be of serious consequence in flocks evidently or apparently in poor health as a result of mismanagement and malnutrition as well as parasitic and infectious diseases. On the other hand, the use of pigeon-pox virus, thru virtue of the milder vaccination response induced in chickens, has not been reported to involve the risk of serious sequelae. Altho Glover (1931) reported that pigeon virus may, under unfavorable conditions, produce comb and diphtheritic lesions in chickens, the possibility that the lesions observed represented response to concurrent fowl-pox virus infection does not seem to have been entirely excluded. Bierbaum (1935) stated the belief that pigeon-pox virus never produces post-vaccination secondary infection in chickens. In this connection recent observations (Brandy and Dunlap, 1939) with a single strain of pigeon-pox virus indicate that massive doses of the bacteria-free virus may be given intravenously to three- to four-month-old chickens without significant effect on weight gains or risk of secondary reactions.

The chicks used for comparing the effects of pigeon-pox and fowl-pox viruses were three weeks old. They were the survivors of a large group fed a heavy dose of *S. pullorum* culture when they were one day old. At the time of vaccination a considerable number manifested evidence of some degree of pullorum infection. A total of 330 chicks were divided into three equal lots. One lot was vaccinated by having a 1-percent aqueous suspension of active fowl-pox virus applied to the upper thigh by means of the stick method (two punctures). Another lot was treated in the same manner with a 1-percent suspension of the Illinois strain of pigeon virus. The third lot constituted the control group. The vaccination takes were moderate to severe in the birds treated with fowl-pox virus but mild or questionable in those treated with pigeon virus. Each group was kept isolated but under comparable conditions. Observations were continued for five weeks following vaccination. The data on mortality are included in Table 9.

Because of the high mortality in the fowl-pox group (75.4 percent), no attempt was made to expose any of the surviving chicks to an immunity test. The high mortality in this group illustrated the imminent danger of superimposing fowl-pox virus upon pullorum infection in young chickens. The small difference in the death rates of the pigeon-virus group (10.0 percent) and the control group (9.09 percent) would seem to indicate that the application of pigeon virus by the stick method to broods of young chicks harboring pullorum disease did not appreciably affect their livability. From this experiment it is, of course, impossible to appraise the possible effect of a more satisfactory take or reaction to the pigeon virus, but experience suggests that a severe general reaction would be unlikely to occur. Nevertheless, the rather general distribution and prevalence of pullorum infection in baby chicks, in so far as it bears on the effect of fowl-pox-virus vaccination, cannot be disregarded. This circumstance may warrant caution in the application of recommendations such as those by Sherwood (1932) and Dunn and Sherwood (1933) on vaccination of day-old chicks and turkeys. However, the recent comprehensive studies of Lubbehusen, Beach, and Basic (1936), and Lubbehusen and Beach (1937, 1937A), seem to recognize the influence of devitalizing factors in producing serious post-vaccination losses in baby chicks.

Conclusion. The application of pigeon-pox virus by the stick method did not cause any significant increase in mortality in chicks which survived fed doses of *S. pullorum*. Fowl-pox-virus vaccination applied by the stick method to similar chicks resulted in a high mortality.

SUMMARY

This monograph presents the results of experiments at the Illinois Station testing the effectiveness of different viruses for the control of fowl pox in domestic fowls as well as different methods of applying these viruses. It also includes an extensive review of literature on the subject that had been published up to the time of the writing of the monograph in 1936.

Study of the literature revealed that altho the disease had been described as early as 1869, the virus nature of the causative agent was not recognized until 1902. As early as 1908 it was shown that cutaneous fowl pox and avian diphtheria were different manifestations of the same virus.

In attempts at immunization of chickens against fowl pox the early workers employed principally unmodified fowl-pox lesion material treated by chemical and physical means, with the object of bringing about a satisfactory degree of attenuation. Later virulent unmodified fowl- and pigeon-pox viruses were widely employed. In an effort to

effect desirable modifications of bipathogenic viruses, passage thru the heterologous species was adopted.

Results of studies on the immunizing properties of fowl-pox virus attenuated by heat and by chemicals were variable, but on the whole immunizing power was dependent upon the concentration of living virus remaining in the vaccine. Such vaccines, however, were found undesirable because of their variability.

Working with living unmodified fowl-pox virus, numerous investigators reported that cutaneous vaccination of chickens was in general quite satisfactory. Such vaccination produced a high-grade immunity which persisted for an extended period of time. However, post-vaccination reactions manifested by constitutional disturbances, as well as considerable mortality, were frequently encountered in vaccinated flocks suffering from parasitic and other diseases. Another difficulty was noted in the serious impairment of production following vaccination of laying flocks. This vaccine was therefore recommended only for younger birds. Virus obtained both from comb and wattle lesions, lesions on the chorio-allantoic membranes of developing chick embryos, and tissue culture gave similar results when used as vaccines.

The immunological relation of fowl- and pigeon-pox viruses was first pointed out in 1926. Since that time virulent pigeon-pox virus has been utilized by numerous workers in vaccination against fowl pox. Vaccination with pigeon-pox virus produces no unfavorable reactions, altho the resultant immunity is neither so strong nor so lasting as that produced by fowl-pox virus. The stick method of vaccination was not effective; the feather follicle method was effective.

Modification of fowl-pox virus by pigeon passage and modification of pigeon-pox virus by fowl passage have both been reported to yield more satisfactory vaccines for immunization of chickens.

In the Illinois experiments several thousand young chickens were employed. The results of these experiments may be summarized briefly as follows:

1. Vaccination of young chickens by the feather-follicle method, in which a number of open follicles were treated with an Illinois strain of pigeon-pox virus, resulted in a measurable but incomplete degree of protection against subsequent artificial exposure with fowl-pox virus.

2. The modified protection persisted for a period of two to six months or longer. Chickens vaccinated with pigeon-pox virus by the feather-follicle method and 21 days later placed in contact with affected birds showed no evidence of the disease over a subsequent period of 30 days.

3. Vaccination of chickens with pigeon-pox virus was without severe systemic reactions or other undesirable sequel.

4. Vaccination of young chickens with pigeon-pox virus by the single-stick method failed to produce a measurable resistance against

subsequent artificial exposure with fowl-pox virus. This failure is attributed to the mildness of the inoculation and the reaction compared with the greater quantity of inoculum and the increased degree and extent of the reaction resulting from the feather-follicle method of application.

5. Antidiphtherin of De Blicck and Van Heelsbergen was found to be similar to the Illinois strain of pigeon-pox virus. It was easily propagated upon the skin of the plucked breasts of pigeons, and produced a folliculitis in chickens grossly indistinguishable from that produced by pigeon-pox virus. When antidiphtherin was used in the vaccination of young chickens against fowl pox by the feather-follicle method, it induced a partial immunity similar in degree and duration to that which followed the use of the Illinois strain of pigeon-pox virus.

6. Antidiphtherin applied by the stick method to chickens failed to provide protection against artificial exposure to fowl pox.

7. Vaccination of young chickens by the feather-follicle method with vaccine virus produced folliculitis in some of the chickens but failed to induce an appreciable resistance against artificial exposure to fowl pox.

8. Day-old chicks from pullorum-exposed stock vaccinated with fowl-pox virus by the stick method suffered a mortality of 41 percent within a month following vaccination. Many of these chicks developed generalized pox, and the survivors as a group were stunted and unthrifty.

9. Vaccination of three-week-old chicks with fowl-pox virus by the stick method (the chicks having survived previous exposure to *Salmonella pullorum* in their feed) resulted in a high (75.4 percent) mortality; vaccination of similar chicks with pigeon-pox vaccine by the stick method was not followed by increase in mortality.

10. Both the feather-follicle and the stick methods of vaccinating pigeons with a strain of fowl-pox virus failed to induce a measurable degree of resistance to subsequent artificial exposure with an Illinois strain of pigeon-pox virus.

11. Vaccination of pigeons with a given strain of pigeon-pox virus by the follicle method stimulated a definite immunity against pigeon-pox virus but was often associated with severe systemic reactions.

12. Pigeon pox in the form of powdered scabs, stored at ice-box temperature (10 to 15° C.) for 14 months, lost its pathogenesis and its power to induce either "takes" or resistance against fowl pox when applied to chickens by the feather-follicle method.

13. When the powdered vaccine was stored as 1-percent aqueous suspension at ice-box or room temperature for 31 days, pigeon-pox virus similarly lost its potency.

14. Storage at ice-box temperature for 48 hours of 1-percent suspensions of fowl-pox virus containing different preservatives such as .025 to .5 percent formalin, .5 percent phenol, 2 percent saponin, and .5 percent tricresol resulted in complete loss of viability of the virus and failure to produce either a reaction or resistance to pox in four-week-old chicks.

The practical application of the foregoing findings may be stated as follows:

Potent fowl- and pigeon-pox vaccines properly administered to healthy fowls produce a measurable immunity against fowl pox. The immunity induced by the application of fowl-pox vaccine, stick method, is of longer duration and better defined than the immunity induced by the application of pigeon-pox vaccine by the feather-follicle method. Pigeon-pox vaccine can be used with less risk than fowl-pox vaccine but it provides only a modified protection against the disease. In fact, fowls vaccinated with pigeon-pox vaccine may, upon subsequent exposure, develop lesions, but the systemic disturbance accompanying the unaltered virus infection is appreciably modified and often avoided.

It seems inadvisable to vaccinate laying flocks not exposed to fowl pox with fowl-pox vaccine, tho pigeon-pox vaccine can be employed (feather-follicle method) without exciting harmful effects. On contaminated premises vaccination of fowls 4 to 8 weeks old is recommended. In some cases it may be desirable to precede fowl-pox vaccination by the application of pigeon-pox vaccine. Under many conditions pigeon-pox vaccine may provide a practical degree of protection against natural exposure to fowl pox. The method of application, together with the age of the birds and the general health of the flock at the time of vaccination and immediately thereafter, may largely influence the results.

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